

LC-MS Analysis of Phenolic Acids, Flavonoids and Betanin from Spineless *Opuntia ficus-indica* Fruits

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To cite this article:

Imen Belhadj Slimen, Mahmoud Mabrouk, Chaabane Hanène, Taha Najar, Manef Abderrabba. LC-MS Analysis of Phenolic Acids, Flavonoids and Betanin from Spineless *Opuntia ficus-indica* Fruits. *Cell Biology*. Vol. 5, No. 2, 2017, pp. 17-28.

doi: 10.11648/j.cb.20170502.12

Received: February 16, 2017; **Accepted:** March 11, 2017; **Published:** April 1, 2017

Abstract: *Opuntia ficus-indica* belongs to the Cactaceae family and is widespread in semi-arid and arid regions. Cactus pears are known for their health promoting properties which are due to a variety of bioactive molecules. This study aims to identify and quantify phenolic acids, flavonoids and betanin from spineless *Opuntia ficus-indica* fruits. Fresh mature samples were crushed, and extracted with 50% aqueous ethanol. The identification process was carried out using a Shimadzu high performance liquid chromatograph equipped with a quadrupole mass spectrum. Quantification was made using calibration curves of analytical standards. 9 phenolic acids, 1 flavan-3-ol, 2 flavanones, 3 flavonols, 6 flavones and 2 betacyanins were identified. Important levels of betanin, quinic acid, cirsiolol, acacetin, *trans*cinnamic acid, rutin and naringin were calculated. Our results indicated that *Opuntia ficus-indica* fruits are an important dietary source of phenolic compounds and betalains with high antioxidant capacity.

Keywords: *Opuntia ficus-indica*, Cactus Pear, Polyphenols, Betanin

1. Introduction

Cacti are distinctive and unusual plants especially adapted to extremely arid and hot climates in addition to non-irrigated lands thanks to their CO₂ fixation capacity (Crassulacean Acid Metabolism). Their stems expand into green succulent structures containing the chlorophyll, while the leaves are the spines for which cacti are well known. *Opuntia ficus-indica* is the most important cactus species in agriculture today. It is widely used as food, fodder, dye, source of energy and has role in ecosystem remediation [1].

Opuntia fruits are fleshy and elongated berries, varying in shape, size and color (orange, yellow, red, purple, green, white) and have a consistent number of hard seeds [2]. Their weight varies from 80 g to 140 g, and the average of edible portion is about 54.18% [3]. Cactus pear is a food of

neutraceutical and functional importance. The effect of prickly pear extracts on burned wound, oedema and indigestion was shown to be better than that of stem extracts [4]. Consumption of prickly pears enhances the body redox balance and decreases lipid peroxidation. At comparable levels to those of cactus pear, vitamin C supplementation does not significantly attenuate oxidative stress in humans [5, 6]. Siriwardhana et al. [7] reported an opposite relation between increasing doses of cactus fruit extracts and oxidative-induced DNA damage. Cactus fruits due their functional interest to their content of bioactive molecules. Numerous phytochemicals were identified: phenolic acids, flavonoids, betalains, ascorbic acid, carotenoids and tocopherols. Indeed, prickly pears contain considerable amounts of vitamins, amino acids, sterols, fatty acids, sugars and minerals [8, 9]. This paper aims to identify and quantify

phenolic acids, flavonoids and betalains from Tunisian spineless *Opuntia ficus-indica* fruits.

2. Experimental

2.1. Plant Material

Spineless *Opuntia ficus-indica* fruits were harvested at physiological maturity from the improved pasture managed by the Office of Livestock and Pasture, and located in Sawwaf, Zaghouan governorate (northern Tunisia). Sawwaf has a semi-arid climate, according to the Emberger climate classification. The average annual temperature is 17.8°C and the average annual rainfall is 447 mm. Collected fruits were immediately brought to laboratory and stored at -20°C until use.

2.2. Extract Preparation

The peel was manually removed then fruits were washed, chopped into small pieces, homogenized with aqueous ethanol (1:2; w:v), diluted with distilled water (1:2 v:v) and subjected to a solid-liquid extraction at 4°C for 18h. Homogenates were then centrifuged at 4500g at 4°C for 15min. The supernatants were collected and stored at -20°C until analysis.

2.3. Apparatus and LC-MS Conditions

Chromatography was performed on Shimadzu Ultra Fast Liquid system (Shimadzu prominence UFLC_{XR}, Japon). HPLC separations were performed on an AQUASIL thermo C18 (3×150 mm, 3 µm) at 40°C. An AQUASIL thermoC18 (3×10 mm, 3 µm) guard column was used. Chromatographic separation conditions for phenolic acids and flavonoids were as follow: The mobile phase consisted of water + 0.1% formic acid (A) and methanol + 0.1% formic acid (B). The separation lasted 55 min, followed by 5 min equilibrium time. The binary gradient elution was as follows: 0–45 min, 10% B; 45–55 min, 100% B; 55–55.1 min, 10% B; 55.1–60 min, 10% B. The flow rate was 0.4 mL/min, and the injection volume was 5 µL for analysis. Betanin separation lasted 25 min, with an additional 5 min equilibrium time. The mobile phase consisted of water + 2% formic acid (A) and methanol (B). The binary gradient elution was planned as follows:

0.01–15 min, 5% (B); 15–20 min, 15% (B); 20–22 min, 70% (B); 22–25 min, 100% (B); 25–25.01 min, 100% (B); 25.01–30 min, 5% B. Quantitative determination was carried out using calibration curves of standards.

A Shimadzu 2020 (Japon) Quadrupole mass spectrometer equipped with a positive/negative ESI source was used as a detector. Mass spectrometer was operated in the negative selected ion monitoring (SIM) with capillary voltage at 1.2 V for phenolic compounds identification, and in a positive SIM for betanin. The conditions of MS analysis were designed as follow: the spray voltage was -3.5 V, the desolving line temperature was 250°C, the nebulizer gas flow was 1.5 L/min, the heat block was set at 400°C, the drying gas flow was 12.00 and 15.00 respectively for phenolics and betanin. Finally the detector voltage was 1.2 V.

2.4. Antioxidant Activity

Antiradical activity was measured with DPPH. (1,1-diphenyl-2-picrylhydrazyl radical) as described by Brand-Williams *et al.* [10] and modified by Thaipong *et al.* [11]. The stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and stored at -20°C until use. The working solution was obtained by mixing 10 mL of the stock solution with 45 mL of methanol to obtain an absorbance of 1.1 ± 0.02 units at 515 nm. To evaluate the antiradical activity, 150 µL of each extract was allowed to react with 2850 µL of the DPPH solution for 24h in the dark. The absorbance was taken at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard. The calibration curve was linear between 25 and 800 µM Trolox. Results are expressed as mg TE/g of sample fresh weight.

3. Results and Discussion

3.1. Method Linearity

The linear range of the standard solutions was studied. Calibration curves were linear across a range of concentrations varying from 0.1–20 mg/L. All regression coefficients were higher than 0.99. Calibration curves of quinic acid, catechin (+), hyperoside and rutin are presented in Figure 1.

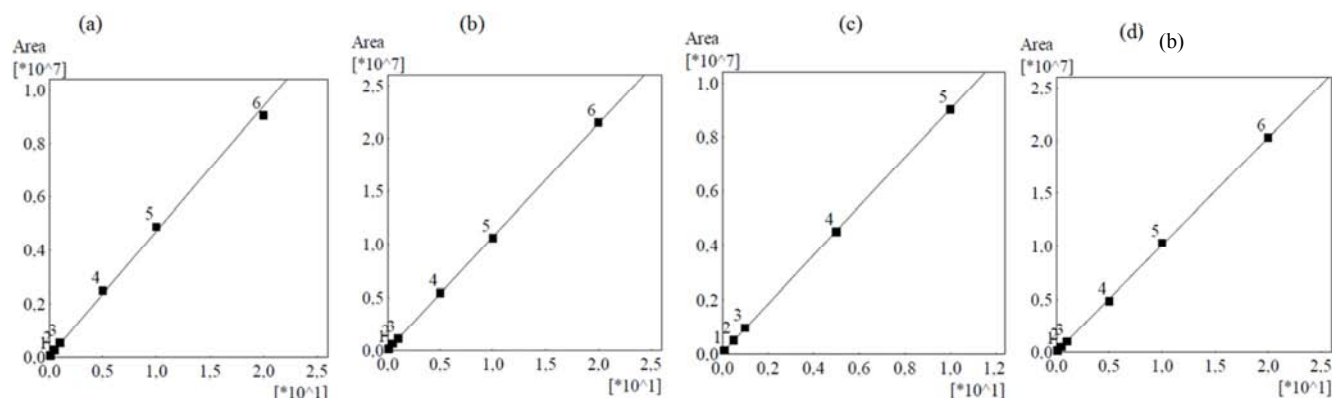


Figure 1. Calibration curves of (a) Quinic acid, (b) Catechin (+) (c) Hyperoside (d) Rutin.

3.2. Identification

High performance liquid chromatography with gradient elution is a widely used technique for the separation and the quantification of phenolic compounds. The ESI source at negative ion mode was selected for the analysis of phenolic compounds while the positive mode was selected for betanin.

Figure 2 shows the total ion chromatogram (TIC) of phenolic compounds from spineless *Opuntia* fruit extract. A total of 21 phenolic molecules in addition to a betanin molecule were identified and confirmed by comparing their MS features and retention times with those of reference compounds. Phenolic compounds include phenolic acids and flavonoids.

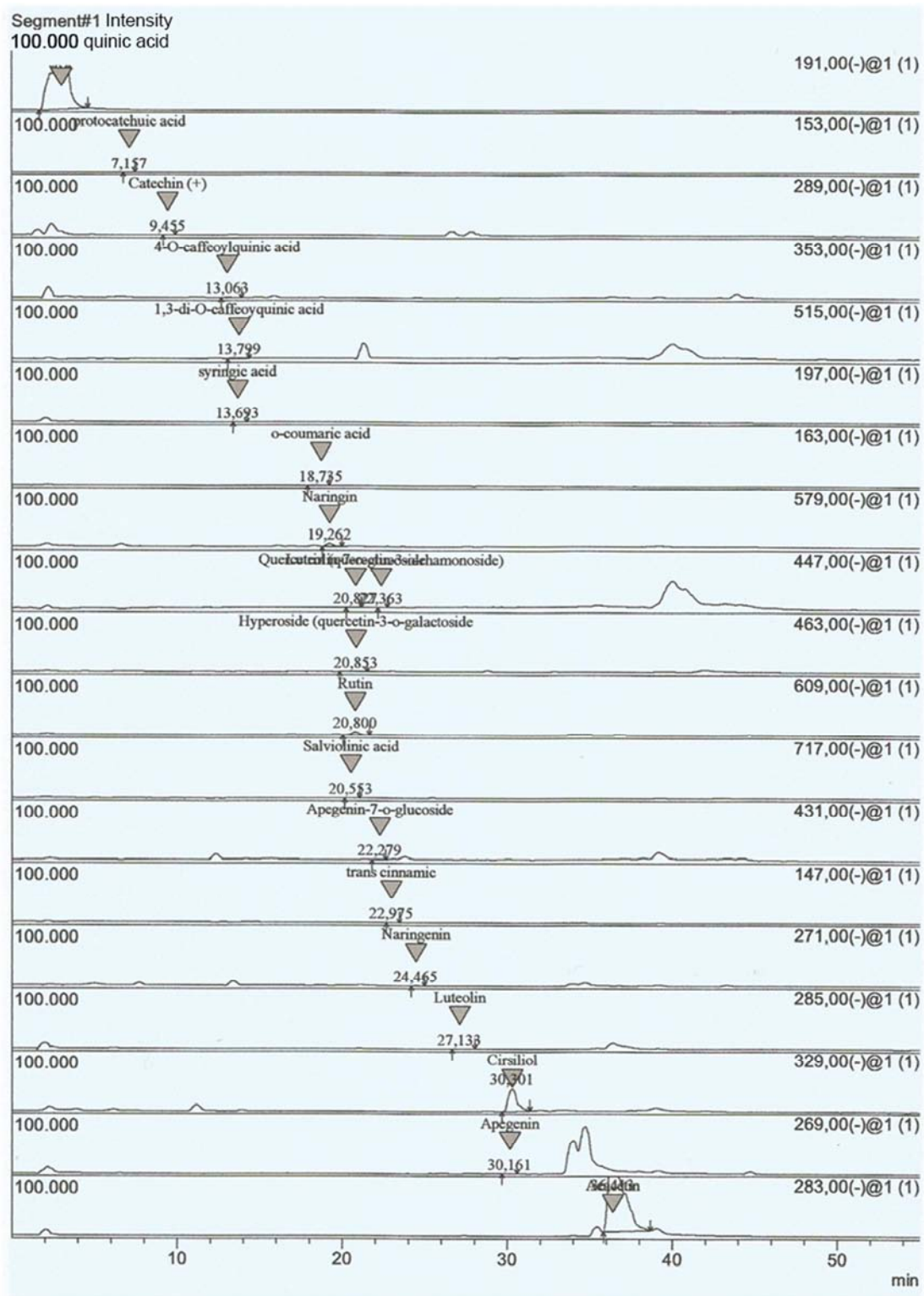


Figure 2. Total ion chromatogram of phenolic compounds from inermis *Opuntia ficus-indica*.

3.2.1. Phenolic Acids

The methodology used allowed the identification of 9 phenolic acids, presented in Table 1. Compound (1) was eluted at 3.01 min and detected at $[M-H]^-$ m/z 191. Compared to the analytical standard, compound 1 was identified as quinic acid (Figure 3). The compound (2) recorded at $[M-H]^-$ m/z 147 at 22.97 min was identified as *trans* cinnamic acid. Based on the fragmentation pattern described by Schaldach and co-authors [12], the fragment ion observed at m/z 119 corresponded to $[M-H-CO]^-$ (Figure 4). Compound (3) showed a $[M-H]^-$ peak at m/z 153 and a retention time of 7.15 min. Compared to the analytical standard, no daughter ions were detected and compound (3) was identified as protocatechuic acid (Figure 5). Compound (4) showed a $[M-H]^-$ peak at m/z 197 at 13.69 min and was identified as syringic acid (Figure 6). Ion of m/z 182 was the major product ion of the parent ion $[M-H]^-$ m/z 197 and corresponded to $[M-H-CH_3]^-$. Compound (5) showed a $[M-H]^-$ peak at m/z 353.00 and a retention time of 13.06 min. No fragment ions were detected and compound (5) was identified as 4-*O*-caffeoylquinic acid (Figure 7). Compound (6) was eluted at 13.79 min and showed a $[M-H]^-$ peak at m/z 515.00. Compared to the standard, it was identified as 1,3-di-*O*-caffeoylquinic acid (cynarin). The fragment ion peak observed at m/z 500 corresponded to $[M-H-CH_3]^-$ and that at m/z 353 corresponded to the chlorogenic acid (Figure 8). Compound (7) showed a $[M-H]^-$ peak at m/z 193.00 and a retention time of 17.31 min and was identified as *trans* ferulic acid. The fragment ion peak at m/z 119 corresponded to $[M-H-CO_2-2CH_3]^-$ (Figure 9). Compound (8) eluted at 18.73 min, recorded at $[M-H]^-$ m/z 163 and identified as *O*-coumaric acid. Finally, Compound (9) recorded at $[M-H]^-$ m/z 717 at 20.55 min was identified as salvianolic acid B (Figure 10).

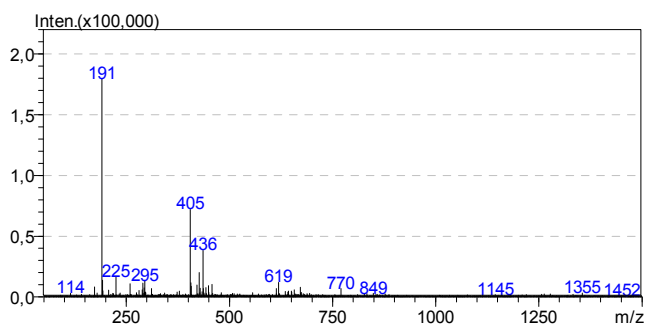


Figure 3. Mass spectrum of quinic acid.

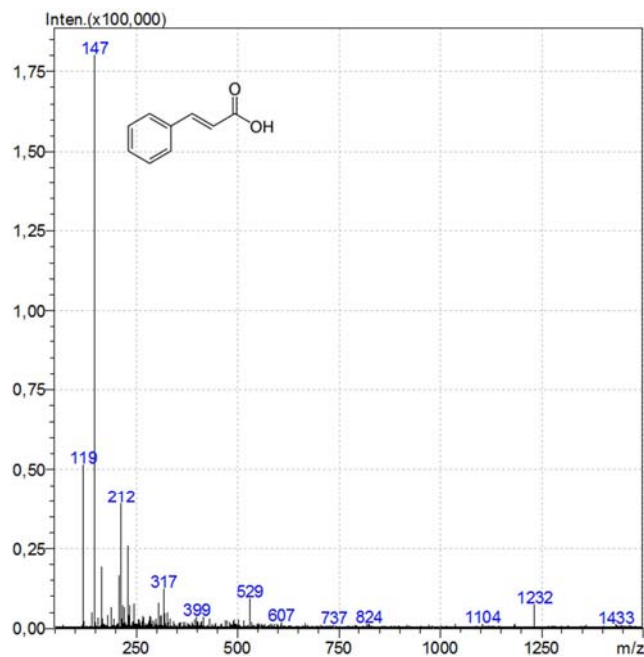


Figure 4. Mass spectrum of *trans* cinnamic acid.

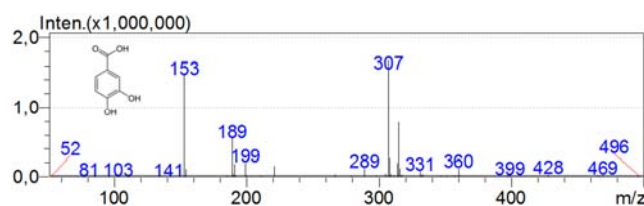


Figure 5. Mass spectrum of protocatechuic acid.

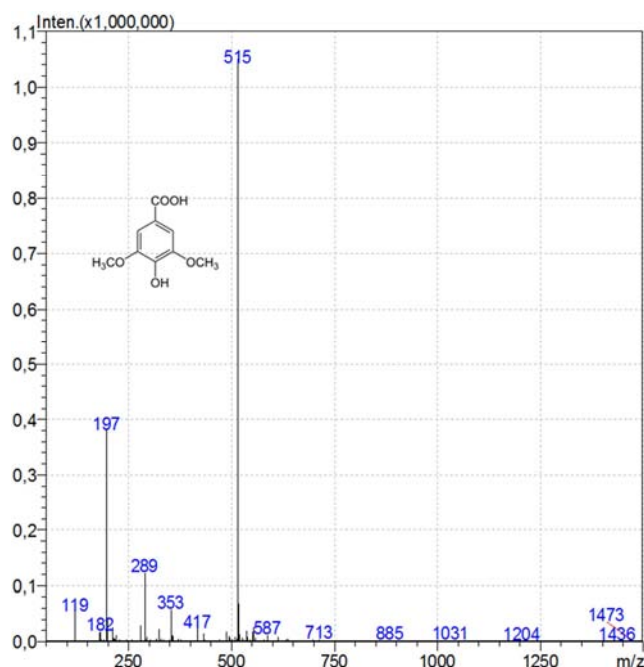


Figure 6. Mass spectrum of syringic acid.

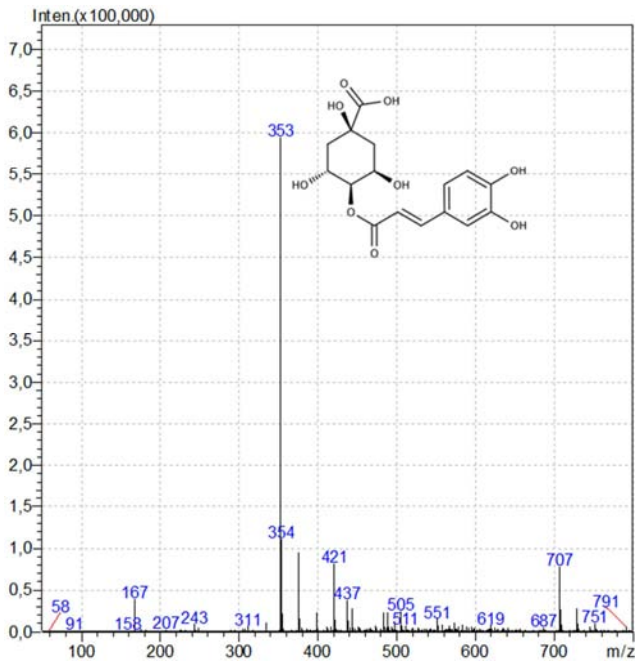


Figure 7. Mass spectrum of 4-O-caffeoylquinic acid.

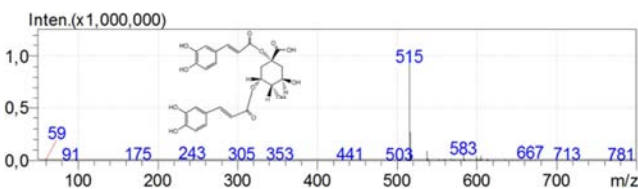


Figure 8. Mass spectrum of 1,3-di-O-caffeoylquinic acid.

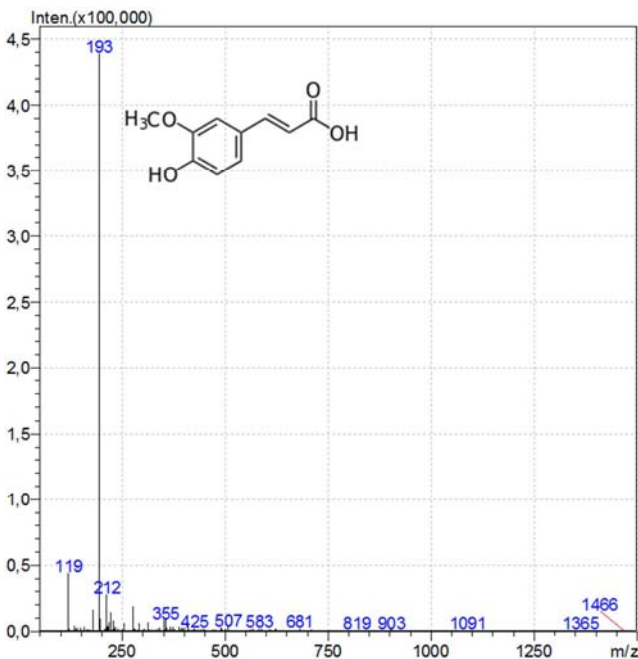


Figure 9. Mass spectrum of trans ferulic acid.

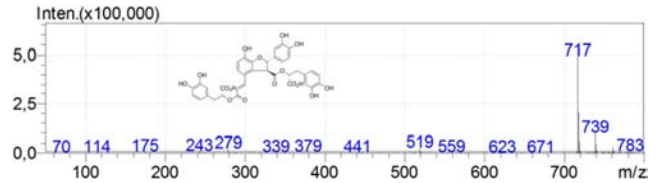


Figure 10. Mass spectrum of salvianolic acid B.

3.2.2. Flavan-3-ol

Compound (10) was eluted at 9.45 min and showed a $[M-H]^-$ peak at m/z 289. A fragment ion was recorded at m/z 245. Based on the MS fragmentation pathway, the retention time and the analytical standard, compound (10) was identified as (+) catechin (Figure 11).

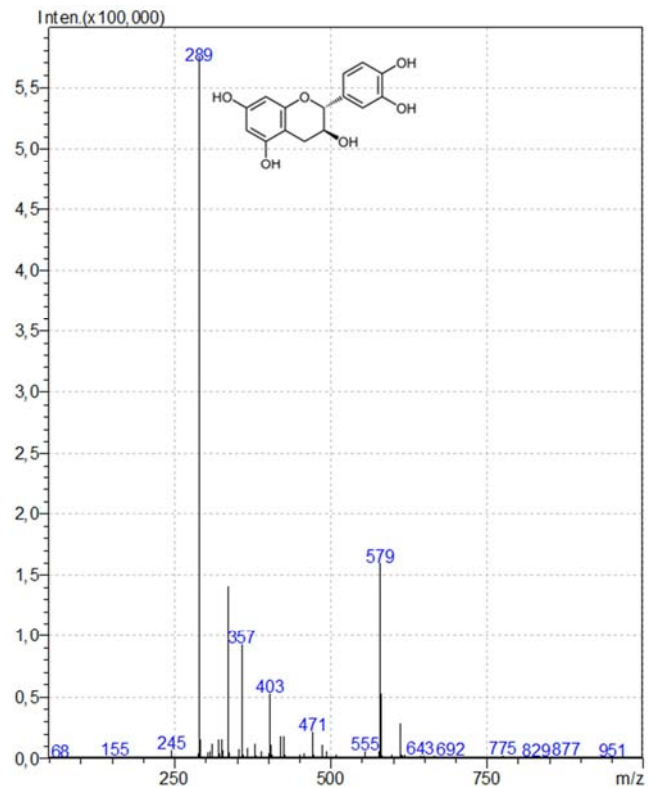


Figure 11. Mass spectrum of catechin (+).

3.2.3. Flavanone

Two flavanones were identified. The first one (compound (11)) showed a $[M-H]^-$ peak at m/z 579 at 19.26 min. Based on the analytical standard mass spectrum, compound (11) was identified as narengin (Figure 12). The fragment ion recorded at m/z 118 may be generated from the completely fragmented precursor ion at m/z 271 which was yielded by the neutral loss of rutinose [13]. The second one (compound (12)) showed a $[M-H]^-$ peak at m/z 271 and a retention time of 24.46 min. A fragment ion was recorded at m/z 175 (Figure 13). Compound 12 was identified as naringenin and its daughter ion was yielded after the loss of the B ring $[M-H - \text{ring B}]^-$ [14].

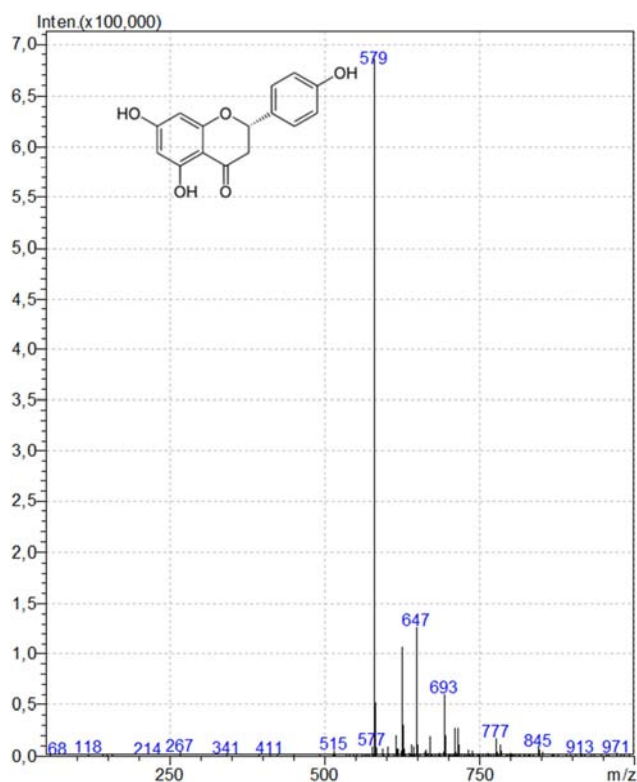


Figure 12. Mass spectrum of naringin.

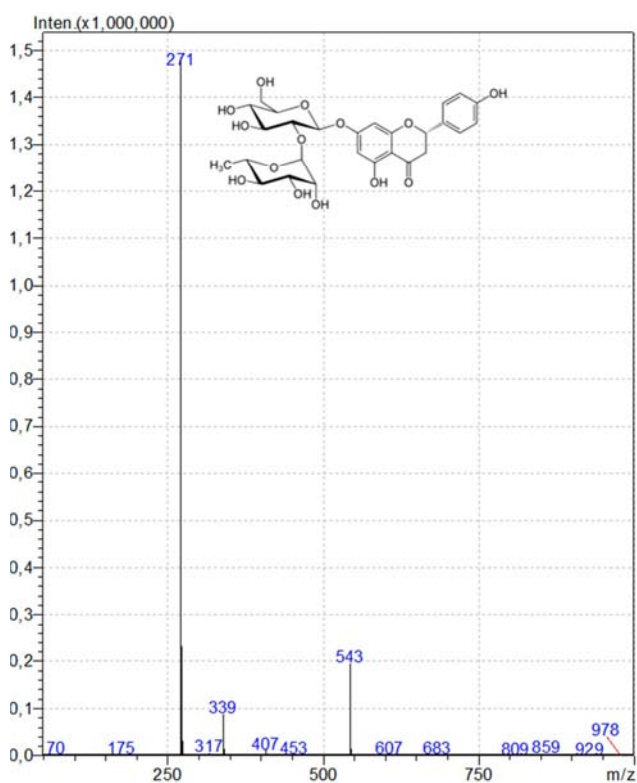


Figure 13. Mass spectrum of naringenin.

3.2.4. Flavonols

In this study, 3 flavonols were identified. Compound (13) showed a [M-H]⁻ peak at m/z 609 at 20.80 min, and was therefore identified as rutin (Figure 14). Compound (14) was eluted at 20.85 min, recorded at [M-H]⁻ m/z 463 and

therefore identified as Quercetin-3-*O*-galactoside (Figure 15). Compound (15) showed a [M-H]⁻ peak at m/z 447 and a retention time of 22.36 min, and was therefore identified as quercetrin. The fragment ion at m/z 301 was formed by the loss of the rhamnose moiety from the glucoside (Figure 16).

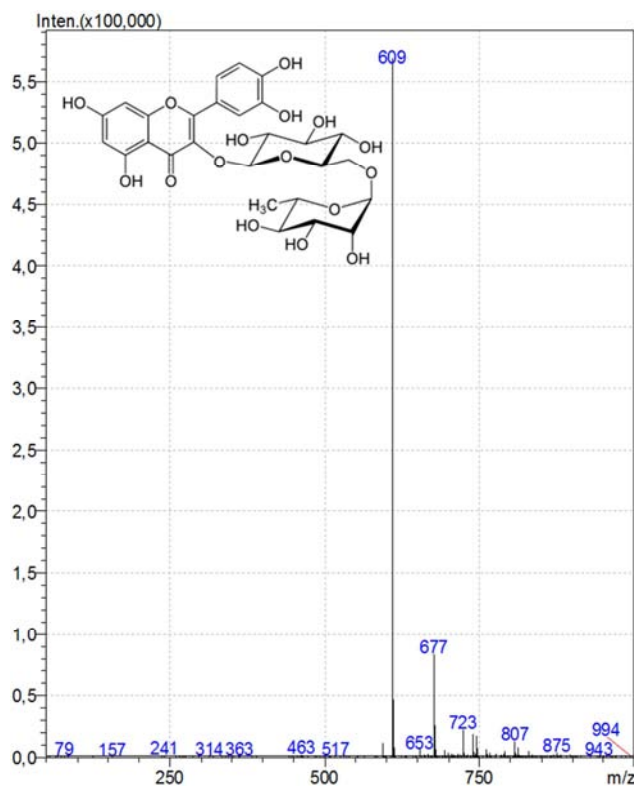


Figure 14. Mass spectrum of rutin.

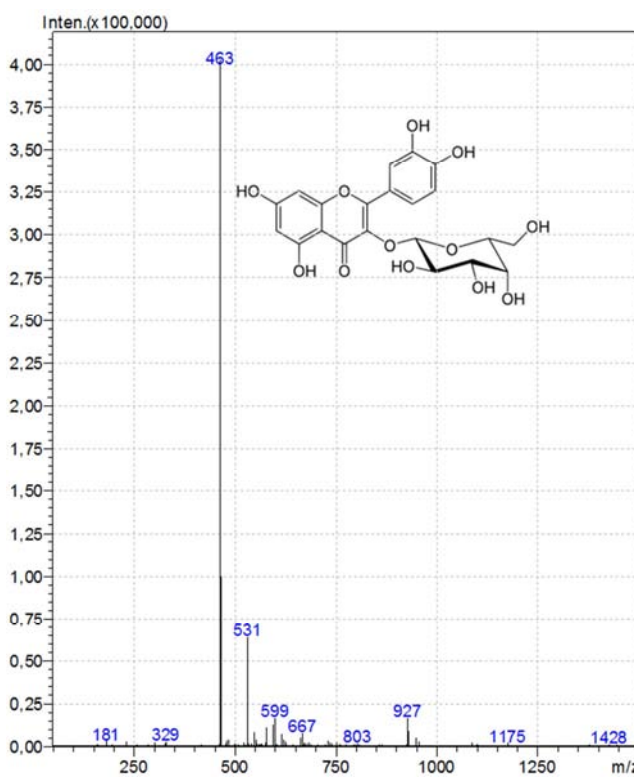


Figure 15. Mass spectrum of hyperoside.

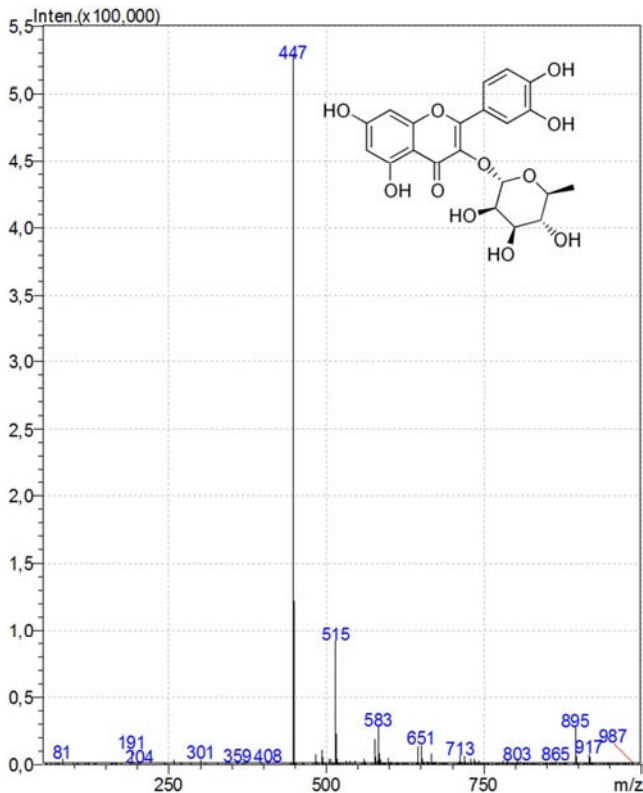


Figure 16. Mass spectrum of quercetrin.

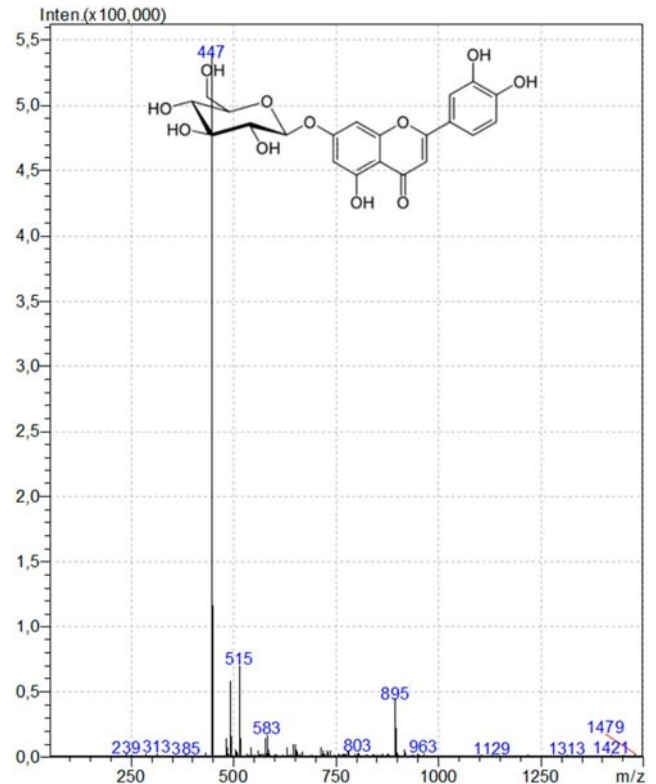
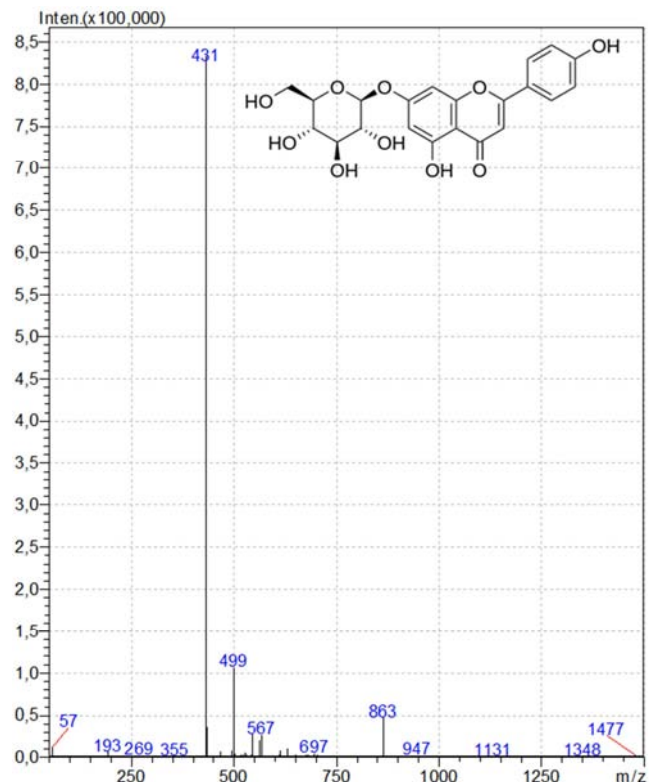


Figure 17. Mass spectrum of cynaroside.

3.2.5. Flavones

In addition to flavonols, 6 flavones were identified in *Opuntia ficus-indica* var *inermis* fruits. Compound (16) showed a [M-H]⁻ peak at m/z 447 at 20.82 min, and was therefore identified as luteolin 7-*O*-glucoside or Cynaroside (Figure 17). Compound (17) showed a [M-H]⁻ peak at m/z 431 and a retention time of 22.27 min, and was therefore identified as apigenin-7-*O*-glucoside. A fragment ion was generated at m/z 269 after the loss of an hexose moiety from the parent ion (Figure 18). Compound (18) was eluted at 27.13 min and recorded at [M-H]⁻ m/z 285, and was therefore identified as luteolin (Figure 19). No fragment ions were detected according to luteolin defragmentation mass spectrum reported by Fabre and collaborators [14]. Compound (19) showed a [M-H]⁻ peak at m/z 269 at 30.16 min, and was therefore identified as apigenin (Figure 20). The [M-H]⁻ fragmentation pathway of this flavone reported by Fabre et al. [14] was not observed. Compound (20) showed a [M-H]⁻ peak at m/z 329 and a retention time of 30.30 min, and was therefore identified as cirsiolol (Figure 21). Compound (21) showed a [M-H]⁻ peak at m/z 283 and a retention time of 36.41 min, and was identified as acacetin. A fragment pic was observed at m/z 268 and corresponded to [M-H-CH₃]⁻ (Figure 22).

Figure 18. Mass spectrum of Apigenin-7-*O*-glucoside.

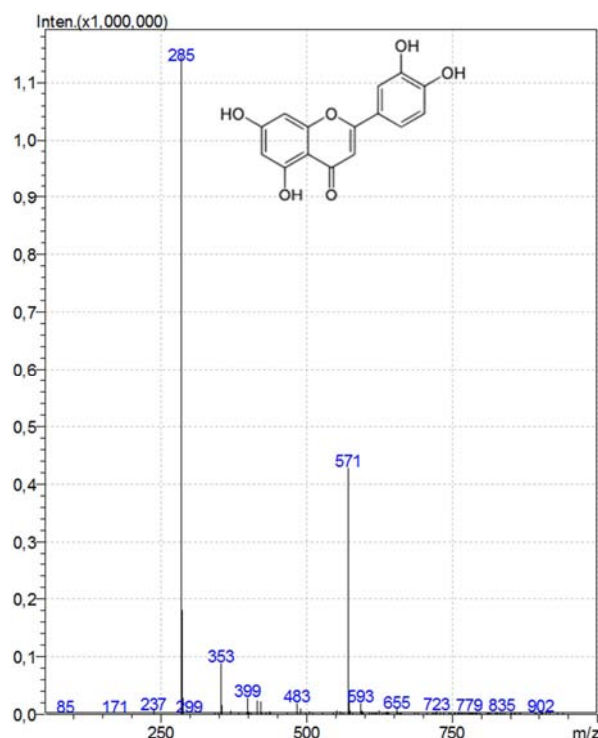


Figure 19. MS spectra of Luteolin.

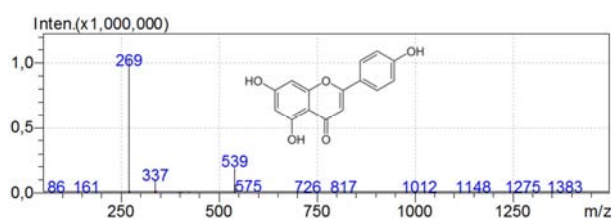


Figure 20. Mass spectrum of apigenin.

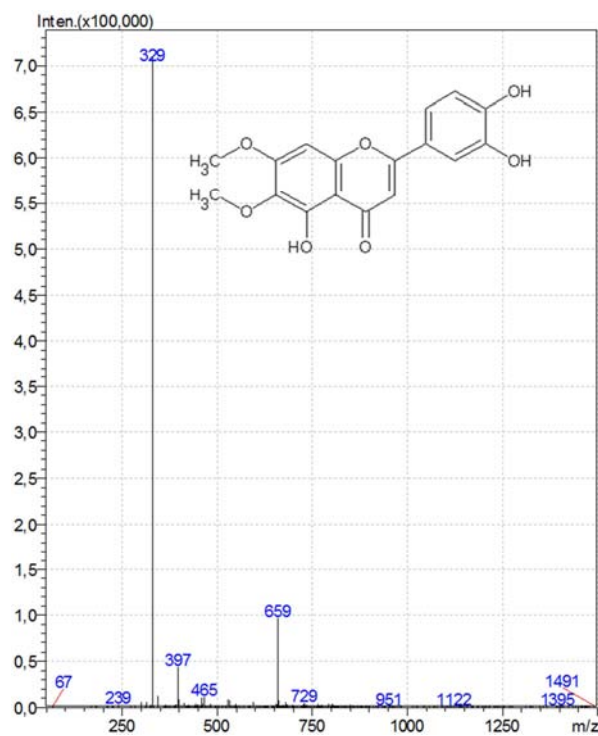


Figure 21. Mass spectrum of cirsiol.

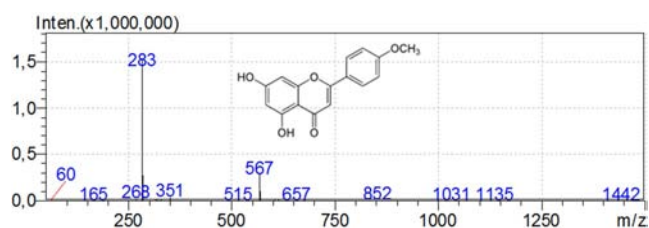


Figure 22. Mass spectrum of acacetin.

3.2.6. Betanin and Betanidin

The molecular ion $[M+H]^+$ peak of betanin was identified at m/z 551. The corresponding retention time was 1.665 min. The mass spectrum (Figure 20) showed a daughter ion at m/z 389. This fragment ion was produced by fragmentation of the parent ion of m/z of 551 assigned to betanin and indicated that it corresponds to the protonated aglycone betanidin obtained by glucose loss. Our results are in accordance with those reported by Belhadj Slimen *et al.* [9] and Nestora *et al.* [15].

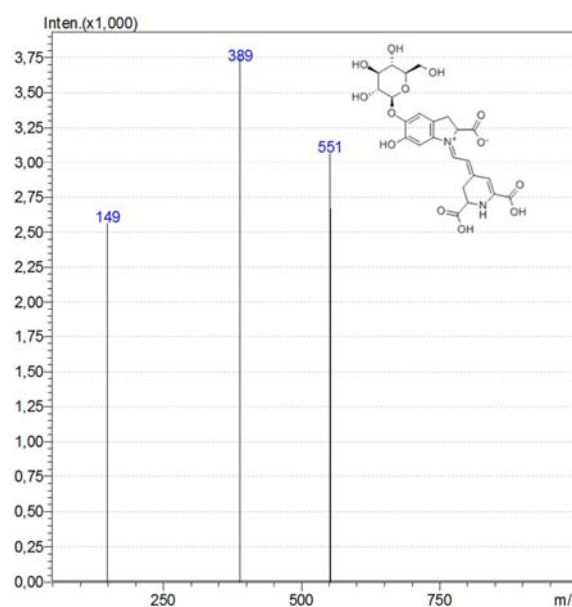


Figure 23. Mass spectrum of betanin.

3.2.7. Undetected Peaks

Undetectable peaks can result from several mechanisms. A compound can be present in a sample, but at low concentration below the detection limit of the spectrometer. It can also be truly absent from the sample. Alternatively, a compound may be present in the sample at a concentration above the detection limit of the mass spectrometer. The technical issues related to sample preparation or processing influence greatly the detection of its compounds. In all cases, a compound should be reported as a missing value in the resultant data set [16].

Undetected compounds are cited in Table 1. Isorhamnetin 3-O-rutinoside was detected in Tunisian *spineless Opuntia ficus-indica* fruits using an LCQ DECA ion trap mass spectrometer equipped with an ESI source and run by Xcalibur software [17]. Quercetin, and Kaempferol were also reported for this species [18, 19].

Table 1. Characterization of phenolic compounds in the *Opuntia ficus-indica* fruits by LC–MS using ESI negative ion mode.

Phenolic compound	Empirical formula	Retention time (min)	<i>m/z</i> peak [M-H] [−]
Quinic acid (1)	C ₇ H ₁₂ O ₆	3.017	191.00
<i>trans</i> cinnamic acid (2)	C ₉ H ₈ O ₂	22.975	147.00
Protocatechuic acid (3)	C ₇ H ₆ O ₄	7.157	153.00
Syringic acid (4)	C ₉ H ₁₀ O ₅	13.693	197.00
4- <i>O</i> -caffeoylquinic acid (5)	C ₁₆ H ₁₈ O ₉	13.063	353.00
1,3-di- <i>O</i> -caffeoylquinic acid (6)	C ₂₅ H ₂₄ O ₁₂	13.799	515.00
<i>trans</i> ferulic acid (7)	C ₁₀ H ₁₀ O ₄	17.317	193.00
<i>O</i> -coumaric acid (8)	C ₉ H ₈ O ₃	18.735	163.00
Salviolinic acid (9)	C ₃₆ H ₃₀ O ₁₆	20.553	717.00
Catechin (+) (10)	C ₁₅ H ₁₄ O ₆	9.455	289.00
Naringin (11)	C ₂₇ H ₃₂ O ₁₄	19.262	579.00
Naringenin (12)	C ₁₅ H ₁₂ O ₅	24.465	271.00
Rutin (13)	C ₂₇ H ₃₀ O ₁₆	20.800	609.00
Hyperoside (quercetin-3- <i>O</i> -galactoside) (14)	C ₂₁ H ₂₀ O ₁₂	20.853	463.00
Quercetrin (quercetin-3- <i>O</i> -rhamnoside) (15)	C ₂₁ H ₂₀ O ₁₁	22.363	447.00
Luteolin-7- <i>O</i> -glucoside (16)	C ₂₁ H ₂₀ O ₁₁	20.827	447.00
Apegenin-7- <i>O</i> -glucoside (17)	C ₂₁ H ₂₀ O ₁₀	22.279	431.00
Luteolin (18)	C ₁₅ H ₁₀ O ₆	27.133	285.00
Apigenin (19)	C ₁₅ H ₁₀ O ₅	30.161	269.00
Cirsiliol (20)	C ₁₇ H ₁₄ O ₇	30.301	329.00
Acacetin (21)	C ₁₆ H ₁₂ O ₅	36.413	283.00
Gallic acid	C ₇ H ₆ O ₅	-	ND
Epicatechin	C ₁₅ H ₁₄ O ₆	-	ND
Chlorogenic acid	C ₁₆ H ₁₈ O ₉	-	ND
3,4-di- <i>O</i> -caffeoylquinic acid	C ₂₅ H ₂₄ O ₁₂	-	ND
4,5-di- <i>O</i> -caffeoylquinic acid	C ₂₅ H ₂₄ O ₁₂	-	ND
Caffeic acid	C ₉ H ₈ O ₄	-	ND
<i>p</i> -coumaric acid	C ₉ H ₈ O ₃	-	ND
Rosmarinic acid	C ₁₈ H ₁₆ O ₈	-	ND
Myricetin	C ₁₅ H ₁₀ O ₈	-	ND
Silymarin	C ₂₅ H ₂₂ O ₁₀	-	ND
Quercetin	C ₁₅ H ₁₀ O ₇	-	ND
Kaempferol	C ₁₅ H ₁₀ O ₆	-	ND
Cirsilineol	C ₁₈ H ₁₆ O ₇	-	ND
Isorhamnetin	C ₁₆ H ₁₂ O ₇	-	ND
Aucubin	C ₁₅ H ₂₂ O ₉	-	ND

3.3. Quantification and Biological Importance

Quantification of phenolic acids, flavonoids and betanin from *Opuntia ficus-indica* fruits was ensured using calibration curves of analytical standards. Total betanin content was evaluated at 1,616 g/Kg. Our result is too much higher than the findings of Tesoriere and co-authors [5] (12.1 mg/Kg) as well as those of Butera and co-workers [20] (10.4 mg/Kg). The observed difference may be due to the ripeness state of the collected samples, their provenance and the harvest season [21-23].

Total phenolics accounted at 481.139 mg/Kg. Respective content of each identified molecule is reported in Table 2. Quinic acid is the major phenolic present in spineless *Opuntia ficus-indica* fruits. It is a cyclic polyol and an important intermediate product in the biosynthesis of aromatic compounds (flavonoids and phenol carboxylic acids) in higher plants, humans and animals [24]. Cirsiliol, acacetin, *trans* cinnamic acid, rutin and naringin were present at lower concentrations (respectively 8.797, 7.191, 4.288, 3.180 and 1.059 mg/Kg). Cirsiliol has previously been isolated from other *Salvia* species [25]. It is a competitive low affinity benzodiazepine receptor ligand [26] known for its sedative and hypnotic effects [27]. It is the most potent inhibitor of

arachidonate 5-lipoxygenase, an enzyme responsible for leukotriene biosynthesis [28]. Acacetin is a 5,7-dihydroxy-4'-methoxyflavone that is broadly distributed in plants, and responsible for many color shades [29]. Acacetin has been reported for its antiperoxidative, antiinflammatory, antiplasmodial and antiproliferative effects [30-33]. In addition, acacetin was reported to inhibit glutathione reductase and cytochrome P450 [34, 35]. Cinnamic acid is an organic acid occurring naturally in plants that has low toxicity and a broad spectrum of antioxidant and biological activities [36]. The antioxidant capacity relative to trolox indicated that the cinnamic acid derivatives are more efficient than their benzoic counterparts [37]. Rutin, also called rutoside, quercetin-3-*O*-rutoside and sophorin, is the glycoside combining the flavonol quercetin and the disaccharide rutinose. Rutin is a highly potent molecule due to its strong antioxidant properties. It was used not only as antimicrobial, antifungal, and antiallergic agent; but also for the treatment of various chronic diseases such as cancer, diabetes, hypertension and hypercholesterolemia. Compared to other flavonoids, rutin is considered as a nontoxic and nonoxidizable molecule [38]. Both naringin and naringenin are strong antioxidants [39, 40]. However, the former is less potent compared with the latter

because the sugar moiety in naringin causes steric hindrance of the scavenging group. Naringin was reported for its favorable effects on obesity, hyperlipidemia, hypertension, cardiac function, hyperglycemia and diabetes, hepatic function, inflammation, oxidative stress and free radical damage [41]. Quercetin, a flavonol with proven health promoting properties, is one of the most potent antioxidants among polyphenols [42, 43]. Two quercetin derivatives (quercetin-*O*-glycosides) were identified in *Opuntia* fruits: hyperoside and quercetrin at respective concentrations 0.234 and 0.033 mg/Kg. Quercetin derivatives can be both lipo- and hydrophilic, depending on the type of substituents in the molecule. Glycosylation of at least one hydroxyl group of quercetin derivatives results in an increase of its hydrophilicity [44]. In addition to quercetin derivatives, we quantified other antioxidant molecules such as protocatechuic acid (or 3,4-Dihydroxybenzoic acid) which is a type of phenolic acid and a major metabolite of antioxidant polyphenols, as well as 4-*O*-caffeoylquinic acid, also known as cryptochlorogenic acid, which is a cinnamic acid derivative and possesses antioxidant properties.

Table 2. Relative concentration of identified phenolic compounds in *Opuntia ficus-indica* fruits.

Molecule	Relative concentration (mg/Kg)
Quinic acid	454.716
Protocatechuic acid	0.127
Catechin (+)	0.074
4- <i>O</i> -caffeoylquinic acid	0.127
Syringic acid	0.852
1,3-di- <i>O</i> -caffeoylquinic acid	0.124
<i>trans</i> ferulic acid	0.006
<i>O</i> -coumaric acid	0.406
Naringin	1.059
Salviolinic acid	0.057
Rutin	3.180
Luteolin-7- <i>O</i> -glucoside	0.057
Hyperoside	0.234
Apegenin-7- <i>O</i> -glucoside	0.018
Quercetrin	0.033
<i>trans</i> cinnamic acid	4.288
Naringenin	0.003
Luteolin	0.014
Apigenin	0.002
Cirsiliol	8.797
Acacetin	7.191

Finally, it is important to note that methanolic fruit extract of *Opuntia ficus-indica* was reported for its important anticancer activity. This extract was shown to induce apoptosis as evident by loss of cell viability, enhanced ROS, alteration in mitochondrial membrane potential due to changes in lipid peroxidation, and increased DNA damage in cancer cells [45]. Hence, it can be concluded that *Opuntia* fruits anticancer activity is due to their ability to modulate mitochondrial functions, which are important key players in stem cell fate [46, 47], and which alteration is strongly associated to carcinogenesis [48, 49].

3.4. Antioxidant Activity

The antioxidant activity of spineless *Opuntia ficus-indica*

fruits was assessed using the DPPH free radical scavenging test, and was evaluated at 0.262 mg Trolox Equivalent/g. This antioxidant activity seems to be related to the extraction solvent, which allowed extracting high amounts of betanin and phenolic acids, but few concentrations of flavonoids. Our findings are in accordance with Jorge and co-authors [50] who optimized the extraction of phenolic compounds from *Opuntia ficus-indica* skin in a reflux system using 45% ethanol.

4. Conclusion

In fruits from spineless *O. ficus-indica* growing in Sawaf, Tunisia, it was possible to detect 38 compounds from which 23 were identified. These compounds included different classes of phenolics, namely phenolic acids and different families of flavonoids (flavan-3-ol, flavanones, flavonols, flavones) as well as betanin and its aglycone betanidin. Besides the quantification of phenolics and betanin from *Opuntia ficus-indica* fruits, the paper devoted a special effort to describe some antioxidant and biological activities of the identified compounds. Our results show that *Opuntia* fruits can offer a high interesting value for food and pharmaceutical industries for which the development of a real cactus-sourcing branch is awaited.

Acknowledgment

The authors thank Pr. Touhami Khorchani, the head of the Central Laboratory of the Institute of Arid Regions (Medenine, Tunisia), for accepting, funding and facilitating our samples analysis in his laboratory.

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