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Comparative Study of Antioxidant Power, Polyphenols, Flavonoids and Betacyanims of Peel and Pulp of Three Tunisian *Opuntia* Forms

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Abstract: The antioxidant activity and the chemical composition of methanol extracts from peel and pulp belonging to two species of Tunisian prickly pears *Opuntia ficus indica* (spiny and thornless forms) and *Opuntia stricta* has been studied. The antioxidant capacity was measured by DPPH radical scavenging activity. The Total Phenolic Compound (TPC) and the total flavonoid content were determined by the Folin-Ciocalteu method and colorimetric method, respectively. The phenolic compounds were identified and quantified by High Performance Liquid Chromatography (HPLC) coupled with an electrospray ionization mass spectrometry (ESI-MS). The results showed that *O. stricta* fruits present the best antioxidant activities than the two forms of *O. ficus indica* while the TPC was more important in *O. ficus indica* than in the *O. stricta* fruits. The peels have higher flavonoids than pulps and the thornless has more flavonoid than the spiny. The RP-HPLC and ESI-MS analysis detected two classes of phenolic compounds and betalain pigments. Isorhamnetin derivatives are the dominant flavonol glycoside identified in *O. ficus indica* (spiny: 65.25 μg g^{-1}; thornless: 77.03 μg g^{-1}) and *O. stricta* peels (19.22 μg g^{-1}).

Key words: *Opuntia ficus indica*, *Opuntia stricta*, cactus fruit, antioxidant activity, phenolic compounds

INTRODUCTION

Several species of cactus pear plants belonging to the Cactaceae family originated from Central America (Mexico). These species became widespread in semi-arid regions of the world which include Tunisia (Gurbachan and Felker, 1998). They are of special interest because they are one of the few crops that can be cultivated in areas which offer very little growth possibility for common fruits and vegetables (Han and Felker, 1997).

Cactus pear fruits have many commercial values. They are highly flavored and have nutritional properties. The fruits are used for the manufacture of food products such as juices (Espinosa *et al*., 1973; Ennouri *et al*., 2006), alcoholic beverage (Bustos, 1981), jam (Sawaya *et al*., 1983) and natural liquid sweetener (Saenz and Sepulveda, 2001). One of the more valuable contributions of cactus fresh fruits to a diet is their vitamin C content. In addition to their nutritional properties, these plants contain biocompounds with several commercial applications. One such compound is betalain, a water-soluble nitrogen-containing pigment. It is found in high concentrations in cactus pear plants (Castellar *et al*., 2003; Sanchez *et al*., 2006) in the form of betalamic acid which is the chromophore common to all betalain pigments (Strack *et al*., 2003) used as natural food colorants (Mizrahi *et al*., 1997; Stintzing *et al*., 2003; Stintzing and Carle, 2006). The most important biocoumpounds in cactus fruit are phenolic compounds (Kuti, 2004) and betacyanins, betaxanthins pigments all of which have potent antioxidant properties (Zakharova and Petrova, 1998; Gentile *et al*., 2004). Phenolic compounds can be defined as substances possessing an aromatic ring, carrying one or more hydroxyl groups, including theirs functional derivates (Andres-Lacueva and Zamora-Ros, 2010). The chemical structure and concentration are very variable and depend on the variety, ripening stages and the kind of tissue of plants (Wallace, 1986). Glycosilated flavonols, dihydroflavonols, flavonones and flavonols have been found in cactaceae plants and fruits (Kuti, 2000). The antioxidant properties of the phenolic compounds in cactus pear plants, makes the fruit an important product for preventing human health against degenerative diseases such as cancer, diabetes, gastric, hypercholesterolemia, arteriosclerosis or cardiovascular

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diseases as reported for other flavonol-rich foods (Jacob *et al*., 2008; Lampila *et al*., 2009; Trejo-Gonzalez *et al*., 1996; Galati *et al*., 2003). The research of Butera *et al*. (2002) showed that the antioxidant activity of cactus fruit was two times higher than the tomatoes, pears, apples, bananas, white grapes and very close to the red grapes.

Kuti (1992) showed that the flavonoid content of *O. ficus indica* fruits were major contributors to the antioxidant activity. Compared to vitamins, flavonoid are more potent antioxidants by their prooxidative effect on deoxyribonucleic acid, protein and lipid (Shahidi *et al*., 1992). Recent studies have shown that Polyphenolic compounds of *O. ficus indica* fruits are able to induce a hyperpolarization of the plasma membrane and raising the intracellular pool of calcium in human Jurkat T-cell lines (Aires *et al*., 2004). Most of the studies made on cactus pear fruits consist of the chemical analysis of (a) The pulp for vitamin C, polyphenols, betalains and volatile constituent (Di Cesare and Nani, 1992), (b) The skin for polyphenols and lipids and (c) The seeds for lipid profiles (El Kossori *et al*., 1998). But little is done to compare the main biocompounds in different species of the cactus pear fruit.

The most abundant species of cactus pear plants in Tunisia are the *O. ficus indica* and the *O. stricta* belonging to the orders Centrospermae, the genus *Opuntia* and subgenus *Platyopuntia* (Russel and Felker, 1987). The *O. ficus indica* fruit, usually eaten fresh and after peeling, varies in shape, size and color (length: 30-65 mm, width: 20-40 mm) and consist of a thick peel and an edible juicy pulp with many hard seeds. The *O. stricta* ripe fruits are much smaller and not suitable for consumption (length: 20-50 mm, width: 15-30 mm). They have a red purple colored pericarp (thick peel) and a fleshy dark purple colored endocarp that contains small yellow and brown seeds embedded in pulp (Castellar *et al*., 2012).

The aim of the present study is to provide new findings about biocoumpounds of the methanol extract of the fruits (peel and pulp) of the Tunisian *O. ficus indica* and *Opuntia stricta*. Previous studies have shown that the *Opuntia* species have a regional specificity. For example, some phenolic compounds have been identified in Tunisian *O. ficus indica* flowers and not exist in *O. ficus indica* flower cultivated in Sicily (Italy). De Leo *et al*. (2010) analyzed the methanolic extracts of *O. ficus indica* flowers grown in Italy, the resulting chromatogram, showing nine peaks, only seven of them were identified as flavonol glycosylated derivatives. The results of Yeddes *et al*. (2012) work provided further information on phenolic acids and revealed more peaks in flavonol glycoside region chromatogram (Eight flavonols were identified).

Following a previous study on chemical composition, this present study reports a comparison of antioxidant activity, polyphenols and flavonoid contents between the small red purple-fruits *O. stricta* and *O. ficus indica* fruits and an investigation on flavonol and betalain profile of Tunisian *Opuntia*.

MATERIALS AND METHODS

Plant material: Fresh and mature pears fruits of two *Opuntia* species, two forms of *O. ficus indica*: spiny (green-yellow peel and yellow pulp) and thornless (green peel and red-purple pulp) and *O. stricta* (purple peel and pulp) were collected in summer 2011(September). The spiny wild form was from the region of Al-Ala in the centre of Tunisia located at 35°36′N (North) latitude, 9°34′E (East) longitude and 450 m altitude. The thornless cultivated form was from pilot cultivar of Bou Argoub region in the north east of Tunisia located at 36°32′N latitude, 10°33′E longitude and 62 m altitude. *O. stricta* was from botanical garden in Tunis located at 36°49′N latitude, 10°11′E longitude and 5 m altitude.

Solvents and standard phenolics: Methanol (CH_4O), acetonitrile (CH_3CN), formic acid (CH_2O_2) and acetic acid (CH_3COOH) requested for chromatographic analysis and Folin Ciocalteu reagent were purchased from Merck Company (Merck, Darmstadt, Germany). DPPH from Sigma Aldrich (St. Louis M.O, USA) Water was purified on a MilliQ system (Millipore S.A., Molsheim, France). The used standards were provided by Extrasynthese S.A. (Lyon, France) and were the following: rutin (quercetin 3-O-rutinoside), myricitrin (myricetin 3-O-rhamnoside), hyperoside (quercetin 3-O-galactoside), kaempferol 3-O-rutinoside, isorhamnetin 3-O-rutinoside, kaempferide (4′-methylkaempferol), rhamnetin (7-methylquercetin), isorhamnetin (3-methylquercetin), quercitrin (quercetin 3-O-rhamnoside) and myricetin.

Sample preparation: *Opuntia* fruits were washed with distillated water, air dried and hand peeled. Both, peel and pulp were freeze-dried and reduced into powders. The ground dried sample (30 mg) was extracted three times with 1.2 mL MeOH: acetic acid (99:1) sonicated in a water bath at room temperature for 15 min and then centrifuged at 3900 rpm for 15 min (Fish Bioblock Scientific). The argon is used to degas the combined supernatants. The mixture was immediately filtered

through polytetrafluoroethylene (PTFE) membrane (0.45 μm) and stored in refrigerator at -30°C. The described procedure is recommended for the total phenolic assay, total flavonoid assay and RP-HPLC and ESI -MS analysis (Guyot *et al*., 2003).

DPPH radical scavenging activity assay: The antioxidant capacity of the methanol extracts was tested by DPPH (1.1-diphenyl diphenyl-2-picrylhyydrazyl) according to method adopted by Yen and Duth (1994). The DPPH method is the most used for the evaluation of the antioxidant properties and antiradical activity of natural products. DPPH is stable free radical in a methanolic solution. An aliquot of the extracts above (10, 20, 40, 80, 180, 380, 580, 780, 980, 1180, 1580, 1980, 2380, 2980 and 3780 μL) was mixed with 2 mL of DPPH, solution of varying concentrations were obtained. The mixtures were vortexed vigorously for 30 sec and then immediately placed in an UV-visible lambda 25 model «Perkin Elmer» spectrophotometer. Scans of the solution were performed at wavelength ranging from 400-800 nm. And the maximum absorbance was read at 515 nm. When DPPH is placed in an antioxidant solution, It's free radical is inhibited by the antioxidant and then an absorbance measurement is performed. The inhibition of free radical DPPH (I%) was calculated as:

$$I(\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 and A_1 are the absorbance values of the blank (all reagents except the test compounds) and of the tested samples, respectively. The I% were plotted against respective concentrations used. The slope of the linear portion of each graph was used to calculate $IC_{50\%}$ which is the concentration when 50% of the antioxidant is reduced. $IC_{50\%}$ values of the extracts were compared to the $IC_{50\%}$ value of a standard antioxidant, Trolox (acide-6-hydroxy-2, 5, 7, 8 tetramethylchroman-2-carboxylique) used as positive control and obtained by the same procedure.

Determination of the total phenolic content: The amount of total polyphenolic compounds was determined according to Folin-Ciocalteu adapted from Singleton and Rossi (1965). A 0.1 mL of methanol extracts were diluted to 0.5 mL with 2.5% (v/v) acetic acid. A 0.25 mL of Folin Ciocalteu reagent (Merck) was added to 0.5 mL of the diluted extract and was allowed to stand for 3 min at room temperature. One microlitter of 200 g L^{-1} Na_2CO_3 solution was added and the volume was adjusted to 5 mL with distilled water. The mixture was then heated at 70°C for 10 min. After cooling and color development, the absorbance was measured at 700 nm with a spectrophotometer UV (Spectrometer Spectra Max 384 PLUS Molecular Devices) using blank samples composed of distilled water and reagents. The amount of polyphenolic compounds is determined by comparing absorbance values of the samples to the absorption values of rutin and Gallic Acid (GA) (mg rutin or GA g^{-1} fresh weight) standards.

Determination of total flavonoid content: Total flavonoid content in the methanol extract was determined by using the colorimetric method (trichloride aluminum method) adopted by Bahorun *et al*. (1996) in *Nigella sativa* extracts with slight modifications. An aliquot of methanol extracts (0.3 mL) was added to a 5 mL volumetric flask, containing 0.45 mL of distilled deionised water. After 5 min, 0.75 mL of 2% aluminum chloride ($AlCl_3.H_2O$) solution was added. The mixture was shaken and allowed to rest for 10 min of reaction. The absorbance was measured at 415 nm versus prepared methanol blank with an UV-VIS spectrophotometer (Spectrometer Spectra Max 384 PLUS Molecular Devices). The concentration of flavonoids was determined by comparing absorbance values of the sample to the absorption values of rutin used as a standard. Results were expressed as equivalent rutin (mg rutin g^{-1} of fresh weight)±SD (standard deviation) with 3 replications.

RP-HPLC and ESI -MS analysis: RP-HPLC analyses were performed using a Surveyor AS Autosampler including a binary Agilent HP 1100 pumping system, a thermostated column oven and a thermostated automatic injection module. The system was coupled to a TSP UV6000 detector UV used in 240-600 nm range. The column (Merck) was a reversed phase column (150×2.1 mm i.d., 3.5 μm, Agilent Eclipse XDB-RP).The injected volume was 4 μL and the column was thermostated at 30°C. The elution solvent was a mixture of solvent A consisting of ultrapure water/formic acid (99.9:0.1,v/v) and solvent B consisting of acetonitrile/formic acid (99.9:0.1,v/v) and the following gradient was applied: initial, 3% B; 0-5 min, 9% B linear; 5-15 min, 16% B linear; 16-45 min, 50% B linear; the gradient was followed by washing and reconditioning of the column. Helium was used for degassing solvents. Two particular wavelengths were used for quantification of polyphenols: 280 nm for phenolic acids, 350 nm for flavonols (Guyot *et al*., 2003). Quantitative determination was carried out using calibration curves of standards. Phloretic acid and hyperoside were chosen as external standards for quantification of phenolic acids and flavonols. ESI-MS analyses were performed in negative

mode for phenolic compounds (molecular fragmentation losses hydrogen). For better identification of the peaks, the positive mode was used for betalains (molecular fragmentation with hydrogen acquisition). These tests were performed on an LCQ DECA ion trap mass spectrometer (Thermo-Finnigan, San José, CA,USA) equipped with an ESI source and run by Xcalibur (version 1.2) software. The parameters were as follows: ion spry voltage, 3.69 kV; capillary voltage, -70.78 V; capillary temperature, 240.4°C; sheath nitrogen gas flow rate, 66.65 (arbitrary units); auxiliary gas flow rate, 3.81 (arbitrary units); scan range of m/z 50-2000. Samples corresponding to collected HPLC peaks were directly introduced into the ESI source by a built-in syringe pump at the 3 μL. For the generation of Ms^n data, the precursor ions were fragmented by helium gas collision in the ion trap by optimizing the collision energy in order to obtain the intensity of the precursor ion close to 10% of the relative scale spectrum.

Statistical analysis: All experiments were the result of three runs that averaged together. The standard deviations were based on triplicate measurements (n = 3). The value for each sample was expressed as the Mean (M)±Standard Deviation (SD). The analyses of variance were performed by ANOVA with software SPSS (version 11.5 for Windows, SPSS Inc). Differences among the means were compared using the Fisher-Snedecor distribution with a level of significance $p<0.05$.

RESULTS AND DISCUSSION

DPPH radical scavenging activity: The antioxidant activity of Tunisian *O. stricta* and *O. ficus indica* fruits methanol extracts of both peel and pulp are compared and shown in Table 1. Results show that there is little difference of the antioxidant activity between the spiny and the thornless *O. ficus indica* (for both peel and pulp). The antioxidant activity of the *O. stricta* and *O. ficus indica* are lower compared to trolox (for both peel and pulp). Results also show that the peel extract for the *O. stricta* has a higher antioxidant activity than the pulp (about 3% higher). But for the *O. ficus indica* the pulp has a higher antioxidant activity than the peel (about 2%). The average antioxidant activity of the peel and the pulp of the *O. stricta* is higher than the average antioxidant activity for *O. ficus indica* (about 13% higher).

Our results are in contradiction with the results of Moussa-Ayoub *et al.* (2011) who used the electron paramagnetic resonance spectroscopy to determine the antioxidant activity. Moussa-Ayoub *et al.* (2011) showed that the *O. ficus indica* (from Sicily/Italy) peel has an antioxidant activity higher than the pulp (about 35% differences). They explained their results by the presence of large amounts of flavonols, phenolics as well as betacyanins in *O. ficus indica* fruit's peel compared to its pulp. Maataoui *et al.* (2006) showed that the purple juice of the *O. ficus indica* has a higher antioxidant activity than the yellow-orange juice. Ammar *et al.* (2012) studied flower extracts at post flowering stage for both *O. stricta* and *O. ficus indica*. Their results show that the *O. stricta* has a higher antioxidant activity than the *O. ficus indica*. All these results indicate that the *O. stricta* has higher antioxidant activity than the *O. ficus indica* regardless of the part of fruit studied. This is possibly related to the darker color of the flower and the fruit.

Table 1: DPPH antioxidant scavenging capacity ($IC_{50\%}$) of two Tunisian *O. ficus indica* forms and *O. stricta* fruit methanol extract and Trolox

	DPPH antioxidant scavenging capacity $IC_{50\%}$ (mg mL^{-1})[a]	
Tissue fruit species-forms	Peel	Pulp
O. ficus indica "spiny"	0.54±0.04[b]	0.51±0.01[c]
O. ficus indica "thoruess"	0.57±0.02[b]	0.56±0.01[c]
O. stricta	0.40±0.03[b]	0.43±0.01[c]
Trolox	0.33±0.01	

[a]Means of triplicate assays (mg mL^{-1})±SD. Means of triplicates in the same column with same letters indicate no significant difference at values $p<0.05$

Total phenolic contents: Polyphenols are an important group of natural compounds, recently considered of the high scientific and therapeutic interest. The Total Polyphenols Content (TPC) was estimated in different methanol extracts (Fig. 1). Results show that the amounts of polyphenols were much greater in the peel than in the pulp. The TPC in the peel of the spiny *O. ficus indica* was 3 fold higher than the pulp. The TPC in the peel of the thornless was 2.21 fold higher than the pulp. Similar results were observed for the *O. stricta*, for which the peel was 1.66 fold higher than the pulp. The comparison between the two cactus pear species shows that the average TPC in the *O. ficus indica* is higher than the average TPC in the *O. sticta* (1.94 fold higher for spiny form and 1.76 fold higher for the thornless form). In conclusion, results show that the TPC is higher in the peel than in the pulp and is also higher in the *O. ficus indica* than the *O. stricta*. These results are in agreement with the study of Moussa-Ayoub *et al.* (2011) and Diaz-Medina *et al.* (2007). All of which showed that the highest concentrations of phenolic compounds in fruits occurred in the skin tissue.

Total flavonoid content: Our work shows that there are much less flavonoids than phenolic compounds in all the cactus pear species studied. The total flavonoid content

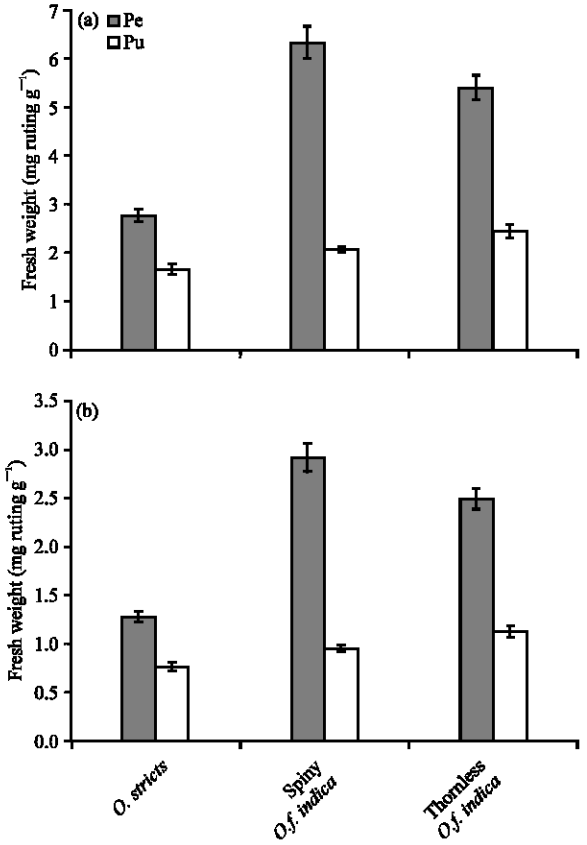

Fig. 1(a-b): Total phenolic content of peel and pulp of three Tunisian *Opuntia* forms (a) Results are expressed as mg rutin equivalents g^{-1} fresh weight and (b) as mg GA equivalents g^{-1} fresh weight (Mean±SD, n = 3), Means of triplicates pulp and pulp indicate no significant difference (level of significance p<0.05) between the three Tunisian *Opuntia* forms

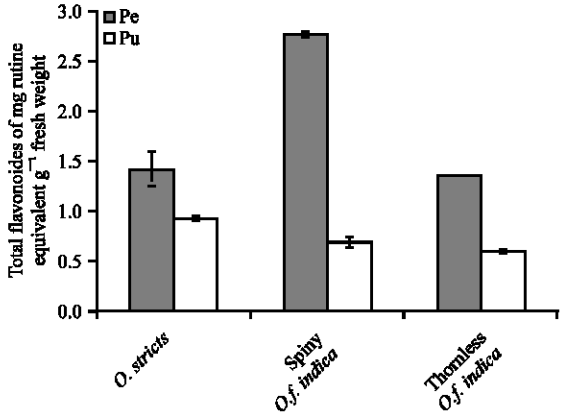

Fig. 2: Total flavonoids content of peel and pulp of three Tunisian *Opuntia* forms, Results are expressed as mg rutin equivalents g^{-1} fresh weight (Mean±SD, n = 3). Means of triplicates pulp and pulp indicate no significant difference (level of significance p<0.05) between the three Tunisian *Opuntia* forms

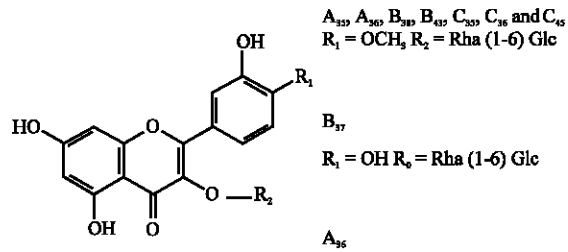

Fig. 3: Chemical structures of flavonol glycosides found in methanol extract fruits of Tunisian *O. ficus indica* and *O. stricta*

is expressed as a concentration in mg of equivalent rutin g^{-1} FW. Results show that the flavonoid content in the peel of the *O. ficus indica* is higher than in the pulp (4 fold higher for the thornless and 2.30 fold higher for the spiny). For the *O. stricta*, the total flavonoid content in the peel is 1.42 fold higher than the pulp. The total flavonoid content of the thornless *O. ficus indica* is higher than the total flavonoid content of the *O. stricta* (1.47 fold higher). The total flavonoid is higher in the thornless *O. ficus indica* than the spiny (1.76 fold higher). In conclusion, the peel contained more flavonoids than the pulp; the thornless contained more flavonoids than the spiny and that the *O. ficus indica* has more flavonoids than the *O. stricta*. These results are in agreement with the work of Ndhlala *et al.* (2007).

It is known that large amounts of polyphenols and flavonoids increase the antioxidant activity. Polyphenols are endowed with potent antioxidant activities *in vitro*, but *in vivo* the scantiness of biomarkers was the major limitation by the lack of robust biomarkers (Chiva-Blanch and Visioli, 2012). The monofloral Cuban honey analysis showed high concentrations of phenolic acids and flavonoids which are responsible, at least in part, for their antioxidant activity (Alvarez-Suarez *et al.*, 2012). But this is contrary to what observed in this study. In this study, we observed, in the pulp of the *O. ficus indica*, a higher antioxidant activity and lower polyphenols and flavonoids (Table 1, Fig. 2-3). Recent studies on the antioxidant activity of *O. ficus indica* and *O. stricta* flowers reported by Ammar *et al.* (2012) showed that during the initial flowering stage the *O. stricta* contains a low amount of total phenolics and a high antioxidant activity. The antioxidant capacity is

determined not only by concentration, but also by several other factors such as the reactivity toward radical, distribution, localization, fate of antioxidant-derived radical, in interaction with other antioxidants (Niki, 2011). In order to evaluate the antioxidant capacity, these factors should be separately assessed and considered. We can attribute the antioxidant activity of the pulp to other compounds and explain our results to Niki (2011) on antioxidant capacity. According to the literature, the content of vitamin C and betalains in *Opuntia* pulp fruits provided higher antioxidant activity and offered a preventive potential against oxidative stress in the human body. Many investigations reported the non-negligible antioxidant activity of betalains due to their redox potentials. Several works have demonstrated the potent antiradical scavenging activity of betalains *in vitro* (Cai *et al*., 2003). In conclusion, the antioxidant activity as shown in this study is not only related to the amount of polyphenols and flavonoids.

RP-HPLC and ESI -MS analysis: Very little work is available in literature on the presence of flavonols in the *O. stricta* fruit. In this investigation we identify and compare amounts of flavonols and other bioactive compounds in the peel and pulp of the Tunisian *O. ficus indica* and the *O. stricta*. The method used in this work is RP-HPLC coupled with UV and ESI-MS. The identification of the compounds (Table 2) was based on

Table 2: RT, spectral, characteristic ions (MS and MS^2) and main amount compounds data from pulp and peel methanol extract of Tunisian *O. ficus indica* and *O. stricta*

Tunisian *Opuntia*	Tissues	Major peaks	Bioactive compounds	RT (min)	MS (m/z)	MS^2 (m/z)	λ_{max} (mn)	Amount (μg g^{-1} fresh weight) (Mean±SD)
Spiny	Peel	A_{31}	Unidentified flavonol	18.45	741	300	352	3.13±0.04
O. ficus indica		A_{32}	Isorhamnetin diglycoside	20.68	769	314-605	355	14.60±0.009
		A_{33}	Isorhamnetin diglycoside	21.05	755	315-605	354	45.59±0.01
		A_{34}	Unidentified flavonol	22.43	609	315-314	263-353	17.48±0.07
		A_{35}	Isorhamnetin 3-O-rutinoside	23.75	623	315-623	352	2.00±0.03
		A_{36}	Isorhamnetin 3-O-rutinoside	24.03	623	315-623	352	1.98±0.002
		A_{37}	Isorhamnetin 3-O-glucoside	24.93	477	314-477	352	1.08±0.01
		A_{38}	Phenolic acid	28.68	431	193-236	328	1.81±0.02
		A_{51}	Indicaxanthin	7.22	309	265	485	n.q
	Pulp	A_{61}	Indicaxanthin	6.98	309	239	485	n.q
Thoruless	Peel	B_{31}	Phenolic acid	12.55	239	239	328	2.44±0.06
O. ficus indica		B_{32}	Phenolic acid	14.00	355	193-355	326	4.69±0.02
		B_{33}	Phenolic acid	15.53	489	235-193	325	8.64±0.03
		B_{34}	Isorhamnetin diglycoside	20.58	769	315-605	355	13.07±0.05
		B_{35}	Isorhamnetin diglycoside	20.95	755	315-605	354	35.09±0.01
		B_{36}	Isorhamnetin diglycoside	21.20	755	315-605	353	10.13±0.02
		B_{37}	Quercetine-3-O-rutinoside	22.35	609	301	350	5.60±0.05
		B_{38}	Isorhamnetin-3-O-rutinoside	23.97	623	315	354	18.74±0.08
		B_{39}	Phenolic acid	28.65	431	193-237	326	5.43±0.04
		B_{51}	Betanin (betanidin-5-O-β-glucoside)	9.52	551	475- 312	530	n.q
	Pulp	B_{41}	Phenolic acid	20.60	613	562-477	270-332	0.34±0.001
		B_{42}	Phenolic acid	20.97	565	339	325	0.75±0.003
		B_{43}	Isorhamnetin 3-O-rutinoside	23.97	623	315	355	0.39±0.005
		B_{61}	Betanin	7.43	551	389	537	n.q
		B_{62}	Isobetanin (isobetanidin-5-O-β-glucoside)	8.43	551	389	537	n.q
		B_{63}	Betanidin	9.63	389	389	541	n.q
O. stricta	Peel	C_{31}	Phenolic acid	12.57	395	349	325	4.15±0.002
		C_{32}	Unidentified compound	16.20	977	815	346	n.q
		C_{33}	Unidentified compound	18.68	639	477	346	n.q
		C_{34}	Unidentified compound	19.27	611	431	345	n.q
		C_{35}	Isorhamnetin 3-O-rutinoside	23.68	623	315	352	4.95±0.01
		C_{36}	Isorhamnetin 3-O-rutinoside	23.97	623	315	355	14.27±0.02
		C_{51}	Betanin	7.72	551	389	533	n.q
		C_{52}	Isobetanin	8.58	551	389	533	n.q
		C_{53}	Betanidin	9.78	389	343-150	539	n.q
	Pulp	C_{41}	Phenolic acid	12.62	395	395-349	326	0.45±0.003
		C_{42}	Phenolic acid	16.08	977	815	330	0.41±0.01
		C_{43}	Phenolic acid	18.58	639	477	330	0.43±0.02
		C_{44}	Phenolic acid	19.17	611	431	334	0.43±0.009
		C_{45}	Isorhamnetin 3-O-rutinoside	23.68	623	315	350	0.85±0.01
		C_{46}	Phenolic acid	24.87	477	314	331	2.00±0.02
		C_{61}	Betanin	7.53	551	389	538	n.q
		C_{62}	Isobetanin	8.40	551	389	533	n.q
		C_{63}	Betanidin	9.87	389	343	525	n.q

M, values expressed as means (μg g^{-1})±SD, standard deviation. n.q, not quantified but identified. Each sample was analyzed in triplicate

a comparison of their HPLC Retention Times (RT), UV-visible spectrum, mass spectrometry (MS and MS^2 fragments) with authentic standards compounds and published data. The betacyanins peaks were identified by order of elution, spectral characteristics and molecular ions $[M+H]^+$ with the red beet methanol extract injected in the same conditions as samples. Accordingly to the available standards, we identified nine chromatographic peaks: A_{35}, A_{36}, B_{38}, B_{43}, C_{35}, C_{36} and C_{45} as isorhamnetin-3-O-rutinoside, A_{36} as isorhamnetin-3-O-glucoside and B_{37} as quercetin-3-O-rutinoside (Fig. 3). All the identified flavonoids belong to the group of flavonol aglycones. Table 2 also shows the quantification of phenolic compounds.

Three types of compounds were detected by UV: Two classes of phenolic compounds (flavonols detected at 350 nm and phenolic acids detected at 280 and 320 nm) and betalain pigments detected at 540 and 470 nm (Table 2).

Flavonol glycosyl: The flavonol glycosil class was indicated by absorbance at 255-263 and 350-355 nm in accordance with the study of Wollenweber (1984). He analyzed the flavonoids in the fronds of *Cheilanthes farinosa* (Polypodiaceae) and detected such peaks at the mentioned wavelength. Results show that the peel of the thornless *O. ficus indica* has the highest amount of phenolic compounds. The most dominant flavonol glycosil are isorhamnetin derivatives. They are found in the peels but not in the pulp (very small amount of isorhamnetin-3-O-rutinoside in the thornless forms and *O. stricta*). The content of these compounds are as follows: thornless *O. ficus indica*: 77.03 μg g^{-1} spiny *O. ficus indica*: 65.25 μg g^{-1} and *O. stricta* 19.22 μg g^{-1}. The peel also contained a compound not found in the spiny *O. ficus indica* and the *O. stricta*. This compound was identified as quercetin 3-O-rutinoside (5.60±0.05 μg g^{-1} FW). Compared to Tunisian fruits that contained more isorhamnetin, less quercetin and no kaempferol, Kuti (2004) showed that the Texas fruit has more quercetin, less isorhamnetin and some kaempferol. Fernandez-Lopez *et al.* (2010) reported for *Opuntia* fruits significant amounts of flavonoids, being quercetin in the predominant type followed by isorhamnetin, luteolin and kaempferol. These results indicate that the phenols are region-specific.

Our MS results showed a signal at m/z 769 (A_{32} and B_{34}). The corresponding MS^2 spectrum exhibited a main product ion at m/z 605 and 314. These results could be correlated to the presence of isorhamnetin (Di Cesare and Nani, 1992). The signal at m/z 605 corresponds to the loss of a fragment with -146 amu (atomic mass unit) in accordance with the mass of a deoxyhexose moiety flowed by one water molecule ($[M-H-146-18]^-$). The fragment at 314 corresponds to the molar mass of an ionic isorhamnetin. The MS spectrum of peaks A_{33}, B_{35} and B_{36} produced a pseudo molecular ion $[M-H]^-$ at m/z 755, releasing a major MS^2 fragment at m/z 605 = [M-H-132-18] and 315, -132 amu is the molar mass of a pentose which loses a water molecule, this allowed us to hypothesize that those signals correspond to an isorhamnetin diglycoside containing one pentose molecule. All these MS results prove the presence of isorhamnetin derivatives.

Phenolic acids: The highest contain of total phenolic acids was found in thornless *O. ficus indica* peel (21.2 μg g^{-1} FW) followed by *O. stricta* peel (4.15 μg g^{-1} FW) and pulp (3.72 μg g^{-1} FW). No phenolic acid were detected in the spiny *O. ficus indica* pulp.

Betalain: The A_{61} showed a pseudo molecular ion $[M+H]^+$ at m/z 309. The corresponding MS^2 spectrum exhibited a main product ion at m/z 239. The A_{61} spectrum (MS and MS^2) was indicative of Indicaxanthin. The MS spectrum of peaks B_{51}, B_{61}, C_{51} and C_{61} produced a pseudo molecular ion $[M+H]^+$ at m/z 551 and the MS^2 spectrum showed produced ions at m/z 389. The peaks were identified as betanin. The chromatographic profile of betalain peel showed the richness of the spiny *O. ficus indica* in indicaxanthin (A_{51} at 470 nm) and the *O. stricta* in betacyanin (C_{51}, C_{52} and C_{53} at 540 nm). The Table 2 revealed a wealth in betacyanin in *O. stricta* and thornless *O. ficus indica* pulp. These results are in agreement with the results of (Castellar *et al.*, 2012) who found that the level of betamin and isobetamin were around five times higher in Murcia *O. stricta* (southeast of Spain) than in red-purple fruits of *O. ficus-indica.*

CONCLUSION

This comparative study of Tunisian *O. ficus indica* and *O. stricta* fruits peel and pulp indicated the presence of biocompounds with possible commercial applications. These biocompounds include polyphenol and betalains, all of which have antioxidant activity. The chemical composition, the amounts and the nature of compounds vary with species and forms (spiny and thornless). Thus have different antioxidant activity. The *O. stricta* has higher antioxidant activity than the *O. ficus indica*. The peel has higher antioxidant activity than the pulp. The high antioxidant activity of the *O. stricta* is related to the high level of betalain pigments, whereas the high antioxidant activity in the peel is related to the high level of TPC and flavonoids. The main polyphenols in *O. ficus indica* peel were flavonols, more precisely

isorahamnetin derivatives. The main polyphenols in the *O. stricta* pulp are phenolic acids. The quercetin 3-O-rutinoside was only found in the spiny *O. ficus indica*. These findings make Tunisian *Opuntia* fruits a promising source of biologically active polyphenolic and betacyanins mixtures. Further investigations need to be done to identify the unknown peaks and compounds. One suggestion would be to compare these peaks to those produced by known standards using other techniques of extraction and identification. This work can be repeated with samples of the cactus fruits collected at different Tunisian regions and seasons to see how the climate and soil affect the results. Another approach would be to change the methods of extractions with different solvents and compositions.

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