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Polyphenolic Extract of Barbary-Fig (*Opuntia ficus-indica*) Syrup: RP–HPLC–ESI–MS Analysis and Determination of Antioxidant, Antimicrobial and Cancer-Cells Cytotoxic Potentials

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Abstract The traditionally derived syrup of *Opuntia ficus-indica* fruit is commonly used in homemade confectionery. Herein, the aqueous-acetone extract prepared from the Tunisian *O. ficus-indica* syrup was investigated. The qualitatively and quantitatively polyphenolic content was analysed using reversed-phase high-performance liquid chromatography–diode array detection (RP-HPLC–DAD) coupled to electrospray ionisation–mass spectrometry (ESI–MS). The extract contained 19.95±2.01 mg phenolics per gram of fresh starting material with isorhamnetin 3-*O*-robinobioside as the major compound (22.76%). The syrup extract showed strong antioxidant potentials as assessed by both ABTS and DPPH functional methods. It exhibited effective antimicrobial activity, particularly against *Staphylococcus aureus* and *Staphylococcus epidermidis* with a minimal bactericide concentration (MBC) of 1.3 mg phenolics/ml. Furthermore, at final concentrations in the range of 41.38–186.25 μg polyphenols/ml, the extract decreased human SH-SY5Y neuroblastoma and 3T3 fibroblast in vitro cell viability in a dose- and time-dependent manner compared to non-treated control cells. The observed effects were significantly ($P<0.05$) high against cancer lines. Extract concentrations higher than 106.43 μg/ml reduced cancer cells viability to 50–60% 1–3 h post-treatment. Further in vivo insight studies should emphasise and validate the herein obtained results.

Keywords LC–ESI–MS · Polyphenols · Syrup · Antioxidant · Antimicrobial · Cancer cells

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Introduction

Biomolecules in fruits and vegetables have attracted a great deal of attention mainly concentrated on their role in preventing acute and chronic diseases. Epidemiological studies have shown that fruit and vegetable consumption reduced the risk of certain human cancer types (Aleksandra et al. 2011). In recent years, the development of more effective and safer agents has been intensively required for chemoprevention of human cancers. Therefore, natural products from plants, mainly polyphenolic biomolecules and their synthetic derivatives, have been expected to play important roles in creating new and better therapeutic agents (Tao et al.

2010). The importance of many plants as natural cheap sources of polyphenols and as food promoting human health is well established. Consistent with this notion, *Opuntia ficus-indica*, a Cactaceae tree native to Mexico and from where it was widespread throughout semi-arid regions, has been used in traditional medicine. It is claimed to be an excellent source of natural oligoelements which may improve human health and nutrition (Hfaiedh et al. 2008). Even though the plant is mainly cultivated for its sweet and juicy fruit known as 'Barbary fig' or 'prickly pear', the pulp of the fruit has shown antiulcerogenic, antioxidant, anticancer, neuroprotective, hepatoprotective and antiproliferative activities (Dok-Go et al. 2003; Galati et al. 2003; Kuti 2004; Sreekanth et al. 2007; Tesoriere et al. 2004; Zou et al. 2005). Agozzino et al. (2005) reported its use for the treatment of gastritis, hyperglycaemia, arteriosclerosis, diabetes and prostate hypertrophy. Moreover, the tree cladodes are utilised to reduce serum cholesterol level and blood pressure for ulcers treatment, rheumatic pain, wounds, fatigue, capillary fragility and liver conditions (Agozzino et al. 2005).

The traditionally fruit-derived syrup, namely 'Rub el Hendy', is commonly used for the preparation of cakes, cookies and homemade confectionery in North Africa. The preparation process consists of mixing the fruit with water (ratio 1:1, w/v) and boiling about 90 min. Following a filtration through a cloth, the juice is collected. In the same way, the remaining press cake is then re-extracted three times with water. The combined collected juice is filtered and boiled down until reaching 65–75 °Bx syrup. This seasonal food by-product is appropriate for conservation for many months and even years since it has low free water content. Barbary-fig syrup is widely consumed all year in Tunisia and other Arabic countries, in replacement to date syrup; it is mainly poured on cooked dough (asseeda) on specific occasions, such as religious festivities (FAO 2004). For its high energetic sugar content, it is frequently consumed during the cold periods of the year.

Limited scientific works on *O. ficus-indica* fruit, cladodes and flowers reported their richness in biofunctional polyphenolic compounds (Kuti 2004; Galati et al. 2005; De Leo et al. 2010; Ncibi et al. 2008). To our knowledge, there is no work on this fruit-derived syrup, particularly its polyphenolic content and related biological potentials. Hence, the objective of this study is to identify and quantify the phenolic profile (LC–DAD–MS) of the Tunisian traditionally made *O. ficus-indica* syrup. The biofunctional potentials of this extract are also evaluated in terms of antioxidant and antimicrobial activities in comparison with well-established reference substances. Also, the effect of the extract on the in vitro viability of human tumourigenic and non-tumourigenic cells is investigated.

Materials and Methods

Chemicals and Reagents Polyphenolic standards, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)], Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the nutrient mixture HAM were from Sigma-Aldrich (France). Folin–Ciocalteu reagent (100%), methanol and formic acid were from Fluka (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and antibiotics were purchased from Gibco BRL (Grand Island, NY, USA). All phenolic standards (caffeic acid, isorhamnetin 3-*O*-glucoside, isorhamnetin 3-*O*-robinobioside, isorhamnetin 3-*O*-galactoside, kaempferol 3-*O*-arabinoside, kaempferol 3-*O*-rutinoside, quercetin 3-*O*-rutinoside, quercetin-dicoumaryl glycoside) were prepared as stock solutions in methanol. Working standards were made by diluting stock solutions in methanol to yield concentrations ranging between 0.05 mg/l and 20 mg/l. Stock/working solutions of the standards were stored in darkness at −20 °C. Water was distilled and filtered through a Milli-Q apparatus before use.

Syrup Material Three traditionally made *O. ficus-indica* syrup samples were purchased from a local market in Hammamet (north-east of Tunisia). The investigated syrups have roughly 74±1 °Bx as determined using a digital refractometer (0–85 °Bx; ATAGO®, Tokyo, Japan). In the laboratory, samples were divided in aliquots (50 g) and frozen at −20 °C until used.

Polyphenols Extraction The syrup (1 g) was mixed with 3 ml of water, vortexed vigorously and sonicated for 20 min. Then, 7 ml of cold acetone (−20 °C) was added to the mixture. Following a centrifugation at 10,000×*g* for 15 min, the residue was re-extracted twice again with 5 ml acetone (−20 °C). The supernatants were collected, pooled and concentrated using a rotary evaporator (60 °C) to a final volume of 3 ml (Dhaouadi et al. 2011). To prevent oxidation of the polyphenols, extraction was rapidly achieved and extracts were immediately used or stored in darkness at −20 °C until further use.

Colorimetric Estimation of Total Phenolic Content Prior to HPLC analysis, the total phenolic content (TPC) was estimated spectrometrically using the Folin–Ciocalteu assay described by Najjaa et al. (2011) with slight modifications. The assay was conducted by mixing 50 µl of deionised water, 50 µl of extract and 400 µl of Folin–Ciocalteu reagent (10%). Following incubation for 10 min in the dark at room temperature, 500 µl of 10 % Na_2CO_3 was added and the mixture was incubated for 1 h. The absorbance at 765 nm was measured using S-22 UV/VIS spectrophotometer

(BOECO, Germany). The TPC was measured in triplicate. Results were expressed as gallic acid equivalent (GAE) in milligrams per gram of the starting syrup.

Reversed Phase HPLC–DAD The aqueous-acetone extracts of the three traditionally made *O. ficus-indica* syrup samples were analysed by means of RP-HPLC. Phenolic compounds were separated using the previously described liquid chromatography technique (Fattouch et al. 2007) with minor modifications. A HPLC system (Bruker Daltonics GmbH, Germany), having a diode array detector model D166, an L-7200 auto sampler and an L-7100 pump (Merck), was used. Data were processed with a Merck-Hitachi D-7000 Chromatography Data Station Software. The separation was achieved on a Merck LiChrospher 100 RP-18 (5 μm, 25×4 mm) column at room temperature. The mobile phase comprised (A) 1% formic acid in Milli-Q water and (B) methanol, which were previously degassed with a sonicator system. The solvent gradient started at 95% A and 5% B, reaching 75% A at 10 min, 65% A at 30 min, 55% A at 35 min, 55% A at 40 min, 50% A at 45 min, 25% A at 50 min, followed by a post-time isocratic plateau for 10 min at 95% A before the next injection. The flow rate was 0.5 ml/min, and the injection volume was 10 μl. The monitoring wavelength was 280 nm.

LC–Electrospray Ionisation–Mass Spectrometry The LC–ESI–MS system consisted of an Agilent LC 1100 series (Agilent Technologies, Inc., CA, USA) controlled by the Chemstation software. The HPLC instrument was coupled to an Esquire 3000+ (Bruker Daltonics GmbH, Germany) mass spectrometer equipped with an ESI source and ion trap mass analyser. The ESI was operated in the negative mode with ESI source probe at 250 °C, CDL at 250 °C, block at 240 °C, flow gas (N_2) at 4.5 l/min, probe voltage 4.5 kV, fragmentor voltage 20 V and a nominal mass range up to m/z 800.

Antioxidant DPPH Method Free radical scavenging activity was determined spectrophotometrically according to the slightly modified method of Najjaa et al. (2011). The $DPPH^{\bullet}$ radical is reduced when reacting with an antioxidant compound, which can donate hydrogen. The polyphenolic extract (25 μl) was placed in a cuvette with 975 μl of 0.04 mM methanolic solution of DPPH radical. The changes in colour (from deep violet to dark yellow) were measured at 517 nm after 1 h. All determinations were performed in triplicate. The DPPH inhibition percentage was calculated as the absorbance decrease of the antioxidant samples (at 1 h) relative to the control. The Trolox equivalent antioxidant capacity (TEAC) was calculated from the equation determined from linear regression after plotting known solutions of Trolox (50–800 μM). The antiradical activity was also expressed as the DPPH inhibition percentage as calculated using the following formula:

$$\% \text{ inhibition} = [(\text{control OD} - \text{sample OD})/\text{control OD}] \times 100.$$

Antioxidant ABTS Assay The antiradical activity was also assessed using a second functional test based on the $ABTS^+$ scavenging potential as described by Tuberoso et al. (2007). Briefly, the $ABTS^+$ radical was generated by reacting 7 mM ABTS and 2.45 mM potassium persulphate. After incubation at room temperature in the dark for 16 h, the solution was diluted to get an absorbance of 0.70±0.02 at 734 nm. The $ABTS^+$ solution (1 ml) was added of the test sample (50 μl), mixed thoroughly and incubated for 30 min. The absorbance of the reactive mixture was measured at 734 nm and compared to the antioxidant potency of Trolox used as a reference. The results were expressed in terms of TEAC.

Antimicrobial Assay In order to evaluate the in vitro antibacterial activities of the prepared extracts, a range of microorganisms was used: the $Gram^+$ *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 106510), *Bacillus cereus* (ATCC11778), the $Gram^-$ *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Salmonella* sp. strain isolated from food and the yeast *Candida albicans* (ATCC 14053). All microorganisms except *C. albicans* were grown at 37 °C for 24 h in nutrient broth. Susceptibility of the test organism to the extract was determined by employing the standard disk and well diffusion techniques. The bacterial suspension in broth, adjusted to 0.5 McFarland turbidity and evaluated using a serial 10-fold dilution method, was spread plated on count agar Muller Hinton (MH) medium in order to give a population of 10^8 colony-forming units (cfu) per plate. For the disk diffusion test, sterile paper discs (6 mm Ø) were added of the test sample (20 μl) and placed onto the inoculated agar surface. After cultivation at 37 °C (bacteria)/27 °C (*C. albicans*) for 24 h, the resulting inhibition zones diameters were measured. Using the well (6 mm Ø) diffusion technique, 70 μl of the sample was tested. Six separate experiments have been carried out at two different times (three for each time).

The minimum inhibitory and bactericidal concentrations (MIC and MBC) were determined using the broth microdilution method (Koneman 1995). A 100 μl amount of a given dilution of the polyphenolic extract and 100 μl of the bacterial suspensions (5×10^5 cfu/ml) were added in the microwells. The plates were incubated aerobically at 37 °C for 24 h. Bacterial growth was revealed by the presence of turbidity and a ‘pellet’ on the well bottom. MICs were determined as the first well in ascending order that did not produce a pellet. To confirm MIC and establish MBC, 25 μl of the broth was removed from each well and inoculated on MH plates. After overnight incubation at 37 °C, the number

of surviving organisms was determined; MBC was determined when 99.9% of bacteria were dead (Fattouch et al. 2007). Six separate experiments have been carried out at two different times (three for each time).

In Vitro Human Cell Viability Assay Human tumourigenic neuroblastoma SH-SY5Y and non-tumourigenic 3T3 fibroblast cells were obtained from the European collection cell. Neuroblastoma cells were cultivated in DMEM/HAM (1:1, v:v) supplemented by 10% heat-inactivated foetal bovine serum (FBS) in the presence of 1% penicillin/streptomycin. Fibroblast cells were cultured in DMEM, 10% heat-inactivated FBS and 1% of mixed antibiotics (streptomycin, penicillin and neomycin). Cells were grown in 75 cm cell culture flasks at 37 °C in a humidified atmosphere containing 5% CO_2 in air (Lantto et al. 2009) until 95% confluence. Cell viability was measured in 96-well plates based on the mitochondrial metabolic activity using the MTT colorimetric assay (Denizot and Lang 1986). Briefly, cells having viability higher than 95% (Sogorb et al. 2010) were added of 200 μl of appropriate diluted syrup extract (41.38–186.25 μg phenolics/ml) and incubated for 1, 3, 6, 24 h at 37 °C. Following extract removal and washing twice with phosphate buffered saline (PBS), 200 μl of MTT (1 mg/ml) was added to the cells and incubated at 37 °C for 3 h. Then, MTT was removed and the coloured formazan was dissolved in DMSO (Barrajon et al. 2010). Using a Bio-Rad 3350 microplate reader, the absorbance (Abs) was determined at 540 nm and at 690 nm as a background. Cell viability (%) was expressed as percentage of the untreated control as follows:

$$\%\,\text{cell viability} = (\text{Abs of treated cells}/\text{Abs of untreated cells}) \times 100$$

Statistical Analysis All tests and analyses were run in triplicate and averaged. Quantitative presented data are means± standard deviations. One-way analysis of variance with Dunnett's post-test was performed using GraphPad Prism version 5.04 for Windows (GraphPad Software, San Diego, CA, USA). Differences of $P<0.05$ were considered significant.

Results and Discussion

Determination of the Total Phenolic Content Barbary-fig fruit has various applications in traditional medicine in many countries besides its usual use as a source of vegetal nutriments. In our study, we are interested to analyse *O. ficus-indica* syrup for its phenolic content, in vitro human cells toxicity, and antioxidant and antibacterial activities. Because of the complexity of various natural mixtures of phenolic compounds, many extraction methods have been reported in the literature using different solvents and a range of selective chromatographic separation steps (Ivanova et al. 2011). In the present work, we used a simple and rapid aqueous acetone extraction method to prepare *O. ficus-indica* syrup phenolic extract. This solvent had been previously shown to provide a good extraction of the main polyphenols from quince peel and pulp material (Fattouch et al. 2007). This procedure permits the elimination of interfering compounds (precipitates proteins and peptides by cold acetone) that could absorb at 280 nm in HPLC analysis. The use of other solvents (methanol, ethanol, water) for the extraction is usually followed by preparative chromatographic SPE or SPME. The reduced number of the cold aqueous-acetone extraction steps should preserve the native bioavailable forms of the phenolics, which is indispensable in the assessment of their biological significance. In order to evaluate the potential biological activities of this product, we first estimated its phenolic content using the Folin–Ciocalteu method. Barbary-fig syrup has a high polyphenolic content of about 34.48±2.6 mg GAE (gallic acid equivalent)/1 g of fresh weight (fw) of syrup. Obtained results are comparable to those reported by Piga et al. (2003) when studying the polyphenolic content changes in cactus pear fruits during 9 days of storage at 4 °C. The difference between the three investigated extracts was not significant ($P>0.05$). The phenolics content of a plant extract may contribute directly to its biological activities, especially the antioxidative action. Accordingly, the antioxidant activities of extracts obtained from plant fruits or their by-products are often explained by their total phenolics and flavonoid contents with good correlations.

Qualitative and Quantitative Analyses The aqueous-acetone extracts of *O. ficus-indica* syrup were then analysed by means of HPLC–ESI–MS technique. In order to reduce the 5% formic acid concentration reported by Fattouch et al. (2007), which may damage HPLC column following repetitive runs, we used this acid at 1 % in the mobile phase (A) and obtained good results. In addition, under the above described conditions, an injection volume of 10 μl of the sample was sufficient to obtain resolvable chromatograms. The chromatographic method allowed the separation and quantification of the phenolic compounds present in the syrup extract. For monitoring all classes of phenolics, no single wavelength is ideal since they display absorbance maxima at different wavelengths, thus the helpful diode array detector was generally recommended. Nevertheless, in our case, 280 nm was satisfactory to detect most syrup phenolics (Fig. 1). The use of DAD allows not only the peak identification but also its purity determination. The purity of all identified peaks in our experiments generally reached 1.00. The obtained chromatogram with 10 peaks (1–10) is shown in Fig. 1. These peaks were identified by comparison of their HPLC retention times (R_T), elution orders and

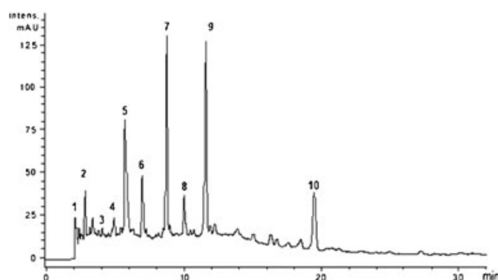

Fig. 1 Typical HPLC profile of *O. ficus-indica* syrup aqueous-acetone extracts with detection at 280 nm. Peaks: *1* ester of ferulic acid, *2* quercetin 3-*O*-rutinoside, *3* kaempferol 3-*O*-rutinoside, *4* quercetin-dicoumaryl glycoside, *5* isorhamnetin 3-*O*-glucoside, *6* caffeic acid, *7* isorhamnetin 3-*O*-robinobioside, *8* ester of coumaric acid, *9* isorhamnetin 3-*O*-galactoside, *10* kaempferol 3-*O*-arabinoside

ESI–MS spectrometric data, with authentic reference compounds (Table 1). Full mass spectra of the observed peaks (1–10) showing deprotonated molecules $[M–H]^-$ confirmed the presence of three different aglycone skeletons, corresponding to kaempferol (*m*/*z*=285), quercetin (*m*/*z*=301) and isorhamnetin (*m*/*z*=315). On the basis of their UV spectra and positive ion masses, peaks 1 and 8 (Fig. 1) seem most likely to be an ester of ferulic acid and an ester of coumaric acid, respectively, as reported in previous studies (De Leo et al. 2010; Galati et al. 2003). All compounds detected in the syrup extract were glycosides carrying one, two or three sugar units and were identified as follows: quercetin 3-*O*-rutinoside, kaempferol 3-*O*-rutinoside, isorhamnetin 3-*O*-glucoside, isorhamnetin 3-*O*-robinobioside, isorhamnetin 3-*O*-galactoside and kaempferol 3-*O*-arabinoside. As determined by the sum of individual amounts of the identified peaks, the total amount of the *O. ficus-indica* syrup polyphenols was 19.95±2.01 mg/g fw. Isorhamnetin 3-*O*-robinobioside was the major component (22.76% of total phenolics), followed by isorhamnetin 3-*O*-galactoside (19.23%), isorhamnetin 3-*O*-glucoside (17.74%) and kaempferol 3-*O*-arabinoside (9.31%). These four compounds represented the main phenolics (69.04%). The free phenolics kaempferol, isorhamnetin and quercetin reported for *Opuntia* fruit, juice and flower (Gurrieri et al. 2000; Galati et al. 2003; De Leo et al. 2010) were not detected in the fruit syrup extract while their glycosylated forms were present. Our results are in agreement with previous reports on the *O. ficus-indica* flower (De Leo et al. 2010) and with studies of its fruit (Galati et al. 2003), where the major constituents were isorhamnetin derivatives. Several pharmacological activities of *O. ficus-indica* were associated to its phenolic compounds as reported for the flowers and prickly pear extracts. For example, Yang et al. (1998) found that the isorhamnetin compound exhibited a testosterone 5 reductase inhibitory potential.

In Vitro Antioxidant Activity The antioxidant activity of Barbary-fig syrup was determined by both anionic (DPPH) and cationic (ABTS) free radicals scavenging potentials. The use of multifunctional tests could be informative to estimate as accurate as possible the real antioxidant potential of the studied biomolecules (Sanchez-Alonso et al. 2007). The difference between ABTS/DPPH based antioxidant activities have been observed for other plant extracts and thought to be correlated with the concentration and chemical structure of individual polyphenolic compounds which could selectively scavenge anionic or cationic free radicals (Butera et al. 2002).

DPPH Radical Scavenging Activity O. ficus-indica syrup extract exhibited a strong $DPPH^•$ scavenging effect of about 3.63±0.12 µmol TEAC/g fw. Our results are comparable to those found by Piga et al. (2003) who analysed different

Table 1 LC–ESI–MS characteristics of the identified polyphenols in the aqueous acetone extracts of the three O. *ficus-indica* syrups (S1, S2, S3)

Peak number	Retention time (R_T)	λ_{max}	$[M–H]^-$	Proposal structure	Content (mg/g fw)				
					S1	S2	S3	Means	SD
1	2.1	242	377	Ester of ferulic acid	NQ	NQ	NQ	–	–
2	2.9	356	609	Quercetin 3-*O*-rutinoside	1.24	1.35	1.46	1.35	0.11
3	4.1	262	593	Kaempferol 3-*O*-rutinoside	0.77	0.17	0.47	0.47	0.30
4	4.8	266	755	Quercetin dicoumaryl glycoside	0.85	0.75	0.65	0.75	0.10
5	5.9	255	477	Isorhamnetin 3-*O*-glucoside	3.97	3.67	4.27	3.97	0.30
6	7.0	242	179	Caffeic acid	1.68	1.80	1.92	1.80	0.12
7	9.1	255	623	Isorhamnetin 3-*O*-robinobioside	5.64	4.66	5.15	5.15	0.49
8	10.2	242	347	Ester of coumaric acid	NQ	NQ	NQ	–	–
9	11.7	252	477	Isorhamnetin 3-*O*-galactoside	4.74	3.96	4.35	4.35	0.39
10	19.5	265	417	Kaempferol 3-*O*-arabinoside	2.11	2.31	1.91	2.11	0.20
Total					21.00	18.67	20.18	19.95	2.01

NQ not quantified, – not calculated

cactus pear fruit during storage period and found that antioxidant activities ranged from 2.3 μmol to 3.1 μmol TEAC/g fw. Since the analysed polyphenolic extracts had high total phenolic content and exhibited strong antioxidant potentials, this should support the application of the cactus fruit syrup in many industrial sectors as a rich source of biofunctional compounds.

ABTS Cation Decolourisation Assay The free radicals scavenging potential of *O. ficus-indica* syrup extract was also assessed by their ability to quench the cationic ABTS+ radical generated in the assay system. Tunisian *O. ficus-indica* syrup extract exhibited 4.70±0.29 μmol TEAC/g fw based on the ABTS method. Insignificant differences (SD<10%) have been recorded between the antioxidant values of the three analysed homemade syrup samples illustrating the slight distinctive process of production.

In Vitro Antimicrobial Effects The consistent and reproducible results obtained using the well and the standard disk diffusion techniques showed that Tunisian *O. ficus-indica* syrup extracts exhibited interesting antimicrobial potentials. The strongest antibacterial activity of the polyphenolic extracts was recorded against *Staphylococcus epidermidis*, whereas the lowest activity was against the *Salmonella* strain. Concerning antifungal test, the analysed extracts failed to show any activity against *C. albicans* (Table 2). The MICs of polyphenols against the six groups of bacteria are listed in Table 2. The values varied from 665 μg/ml to 6,650 μg/ml of culture medium, and the lowest and highest mean MICs among the six groups were observed against *Staphylococcus* species (665 μg/ml) and *P. aeruginosa*/*Salmonella* sp. (6,650 μg/ml), respectively. Barbary-fig syrup extract exhibited also a strong antibacterial activity against *E. coli* (MIC=1,300 μg/ml). The extract showed relative bactericide activities against all tested bacteria except *P. aeruginosa* and *B. cereus* strains. The determined MBC values varied from 1.3 mg to 6.65 mg phenolics/ml with the lowest against *Staph. epidermidis* and *Staph. aureus* and the highest was against *Salmonella* sp. Our results are comparable to Taguri et al. (2004) who tested polyphenolic extracts of 10 different plants against a range of microbial cells and found that Gram(+) were more sensitive than Gram(−) bacteria. *Staph. aureus* [Gram(+)] is known for its high sensitivity to phenolic extracts (Taguri et al. 2004). Generally, Gram(−) are more resistant to bactericidal polyphenols than Gram(+) bacteria. Ikigai et al. (1993) proposed that this difference is caused by repulsion between the phenolics and the surfaces of Gram(−) bacteria, which are coated with lipopolysaccharide. Several studies attributed the inhibitory effect of plant extracts against bacterial pathogens to their phenolic composition (Rodriguez Vaquero et al. 2007). The inhibitory effect of these phenolics could be

Table 2 Antibacterial activity of the *O. ficus-indica* syrup aqueous-acetone extract

		S. aureus (ATCC6538)		*S. epidermidis* (CIP 106510)		*B. cereus* (ATCC1778)		*P. aeruginosa* (ATCC9027)		*E. coli* (ATCC8739)		*Salmonella* sp.		*C. albicans* (ATCC14053)	
		Disk	Well	Disk	Well	Disk	Well	Disk	Well	Disk	Well	Disk	Well	Disk	Well
Diffusion test[a]	Syrup extract	16.9±0.7	19.2±0.5	22.6±0.4	24.7±0.6	19.3±0.5	21.9±1.3	16.3±0.5	18.9±0.7	18.3±0.3	20.3±0.6	12.2±0.4	13.9±1.3	10.3±1.5	12.4±0.7
	AMP	11.2±0.5	15.2±1.3	13.5±1.3	16.3±0.3	19.2±0.7	20.9±0.8	17.5±0.5	18.5±0.6	12.7±1	16.5±1.6	15.7±0.3	17.2±0.3	–	–
	TET	13.5±1.3	17.3±1.1	14.5±1.3	19.5±1.3	18.7±0.6	19.3±0.6	19.8±0.7	20.8±07	14.0±0.7	18.2±0.9	13.5±0.2	15.9±0.5	–	–
MIC[b]		665		665		1.3×10^3		6.65×10^3		1.3×10^3		6.65×10^3		6.65×10^3	
MBC[b]		1.3×10^3		1.3×10^3		3.32×10^3		>		3.32×10^3		6.65×10^3		>	

AMP ampicillin, *TET* oxytetracyclin

[a] Inhibition zone diameter (mm) including the disk or well. Values are mean±SD of six separate experiments carried out at two different times (three for each time)

[b] Phenolic concentration (μg/ml); – no antimicrobial activity (Ø = 6 mm), > no inhibition at the highest reached concentration (6.65×10^3 μg/ml)

explained by adsorption to cell membranes, interaction with enzymes or deprivation of substrate and metal ions (Baydar et al. 2004).

Effect on Human Cell Viability Based on the well-established antioxidant and antiulcerogenic properties of cactus polyphenols, we investigated whether *O. ficus-indica* syrup polyphenolic extract can modulate the in vitro viability of the tumourigenic SH-SY5Y neuroblastoma and non-tumourigenic 3T3 fibroblast cells. Treated with different concentrations of *O. ficus-indica* syrup extract for 1, 3, 6 and 24 h, the cells showed a significant ($P<0.05$) decrease of viability in a dose- and time-dependent manner compared to non-treated control cells. As shown in Fig. 2, the viability of SH-SY5Y cells treated for 1 to 3 h with concentrations of *O. ficus-indica* syrup extract higher than 106.43 μg/ml decreased to 50–60%. At 6 h, neuroblastoma cell viability was significantly decreased more than that of fibroblast. After 24 h of incubation, the viability of the tumourigenic neuroblastoma cells decreased drastically compared to fibroblast cells ($P<0.05$). At 149 μg/ml and after 1, 3 or 6 h of incubation, fibroblast cells maintain viability more than 60%; in contrast, the viability of neuroblastoma cells reached 15% after 6 h of incubation and practically no viability was detected after 24 h (Fig. 2). The obtained data illustrate a distinct behaviour of the tumourigenic and non-tumourigenic cells in the presence of *O. ficus-indica* syrup extract increased concentrations in the medium culture. The susceptibility of the neuroblastoma cells was significantly higher ($P<0.05$) than that of fibroblast cells, suggesting a pronounced cytotoxic effect, particularly against tumourigenic cells. The obtained results emphasise the potential use of the *O. ficus-indica* syrup extract for curative applications. The analysed syrup extract has a pronounced cytotoxic effect on eukaryotic cells, particularly with higher potential against tumourigenic than non-tumourigenic cells. This distinction was central in many scientific studies (Barrajon et al. 2010) to selectively target cancer cells in a therapeutic treatment. Similar results were obtained by Lantto et al. (2009) using SH-SY5Y neuroblastoma and CV1-P fibroblast cells. These authors found that curcumin polyphenolic extracts decreased the cell viability and increased the amount of p53 in SH-SY5Y cells more effectively than in CV1-P cells. Natural extracts have been previously documented as a potential source of anticarcinogenic compounds. In this sense, it is accepted that the chemopreventive and tumour-inhibitory effect associated to some dietary antioxidant polyphenols could be due to their capacity to inhibit oxygen reactive species (ROS) or free radicals. Recently, many studies are evidencing the ability of these compounds to modulate uncontrolled proliferation pathways or protooncogen expression (Menendez et al. 2007). Therefore, it is certainly plausible that the cytotoxicity against cancer cells of these compounds is unrelated to their radical scavenging activity (Friedman et al. 2007). In our case, using the statistical analysis with GraphPad Prism (GraphPad Software version 5.04 for Windows, San Diego, CA, USA), we did not find a significant correlation between cytotoxicity effects of the *O. ficus-indica* extract ($P>0.05$). A recent study on the anticancer activity of several tea extracts with high polyphenolic content has reported CC50 values within the range 0.1–0.5 mg/ml for several cancer cell lines (Friedman et al. 2007). Previous studies on ellagitannins-enriched extracts (Kulkarni et al. 2007) reported CC50 values around 0.55 mg/ml. Therefore, the concentration range of *Cistus* extracts displaying cytotoxicity against M220 (pancreas), MCF7/HER2 and JIMT-1 (breast) cancer cells might be significant to support further studies, especially in MCF7/HER2 and JIMT-1, where a dose response was clearly obtained (Barrajon et al. 2010). The overexpression of HER2 in these cells suggests that *Cistus* extract may be exerting its cytotoxic activity by a HER2-related mechanism as reported before for other polyphenols (Barrajon et al. 2010). Our results indicate a potential role of the studied *O. ficus-indica* syrup extract as health-promoting food constituents as well as candidates for drug development. This study should be followed by a deeper investigation to elucidate the cytotoxic mechanism and to identify the active compounds.

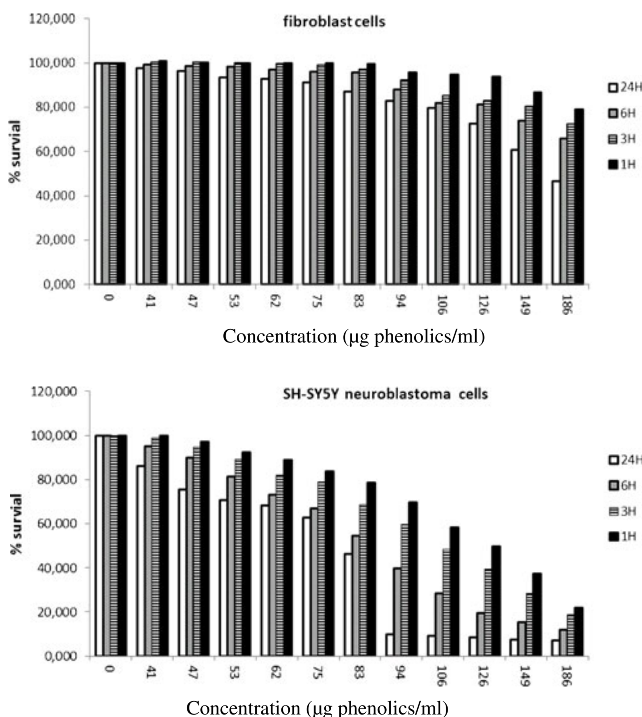

Fig. 2 Viability of the 3T3 fibroblast (*above*) and SH-SY5Y neuroblastoma (*below*) cells treated with different concentrations of *O. ficus-indica* syrup extract. Cell viability was measured by the MTT assay following cells incubation in the presence of the extract during 1, 3, 6 and 24 h. The data are presented as means of three independent experiments (SD<10%) and are different from the untreated control cells at a level of $P<0.05$

Conclusion

This work contributes to the knowledge of the beneficial properties of the phenolic compounds of the traditionally made *O. ficus-indica* syrup. This food-processed by-product could potentially be a good source of natural phenolics with health-promoting effects as well as antioxidant and antimicrobial activities. Even though the role of some polyphenols in the prevention of degenerative diseases such as cancer and cardiovascular diseases is well established, the health-promoting effects of these biomolecules are known to depend on their chemical nature, consumed amount and bioavailability. Studies of cell viability indicate higher sensitivity of neuroblastoma than fibroblast cells. Although the exact mechanisms of the *O. ficus-indica* extract need to be identified, recent reports about other extracts have shown that similar effects can be mediated by specific cellular and molecular mechanisms.

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References

Agozzino P, Avellone G, Caraulo L, Ferrugia M, Flizzola F (2005) Volatile profile of Sicilian prickly pear (*Opuntia ficus*-indica) by SPME-GC/MS analysis. Int J Food Sci 17:341–348

Aleksandra S, Agnieszka T, Edward S (2011) Determination of antioxidant capacity of unprocessed and processed food products by spectrophotometric methods. Food Anal Methods. doi:10.1007/s12161-011-9314-1

Barrajon E, Fernandez S, Saura D, Guillen E, Fernandez A, Segura A, Vicente M (2010) Cistaceae aqueous extracts containing ellagitannins show antioxidant and antimicrobial capacity, and cytotoxic activity against human cancer cells. Food Chem Toxicol 48:2273–2282

Baydar NG, Özkan G, Sagdiç O (2004) Total phenolic contents and antibacterial activities of grapes (*Vitis vinifera* L.) extracts. Food Control 15:335–339

Butera D, Tesoriere L, Digaudio F, Bongiorno A, Allegra M, Pintaudi AM, Kohen R, Livrea M (2002) Antioxidant activities of sicilian prickly pear (*Opuntia ficus* indica) fruit extracts and reducing properties of its betalains:betanin and indicaxanthin. J Agric Food Chem 50:6895–6901

De Leo M, Bruzual M, Pawlowska AM, Cioni PL, Braca A (2010) Profiling the chemical content of *Opuntia ficus*-indica flowers by HPLC–PDA-ESI-MS and GC/EIMS analyses. Phytochem Lett 3:48–52

Denizot F, Lang R (1986) Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Meth 89:271–277

Dhaouadi K, Raboudi F, Estevan C, Barrajon E, Vilanova E, Hamdaoui M, Fattouch S (2011) Cell viability effects and antioxidant and antimicrobial activities of Tunisian date syrup (Rub El Tamer) polyphenolic extracts. J Agric Food Chem 59:402–406

Dok-Go H, Lee KH, Kim HJ, Lee EH, Lee J, Song YS, Lee YH, Jin C, Lee YS, Cho J (2003) Neuroprotective effects of antioxidative flavonoids, quercetin, (+)-dihydroquercetin and quercetin3-methyl ether, isolated from *Opuntia ficus*-indica var. saboten. Progress Brain Res 965:130–136

FAO (2004) Date palm production. www.fao.org/docrep/t0681E/t0681E00.htm

Fattouch S, Caboni P, Coroneo V, Tuberoso CIG, Angioni A, Dessi S, Marzouki N, Cabras P (2007) Antimicrobial activity of Tunisian quince (*Cydonia oblonga* Miller) pulp and peel polyphenolic extracts. J Agric Food Chem 55:963–969

Friedman M, Mackey BE, Kim HJ, Lee IS, Lee KR, Lee SU, Kozukue N (2007) Structure-activity relationships of tea compounds against human cancer cells. J Agric Food Chem 55:243–253

Galati EM, Mondello MR, Giuffrida D, Dugo G, Miceli N, Pergolizzi S, Taviano MF (2003) Chemical characterization and biological effects of Sicilian *Opuntia ficus* indica (L.) Mill. fruit juice: antioxidant and antiulcerogenic activity. J Agric Food Chem 51:4903–4908

Galati EM, Mondello MR, Lauriano ER, Taviano MF, Galluzzo M, Miceli N (2005) *Opuntia Ficus* indica (L.) Mill. fruit juice protects liver from carbon tetrachloride-induced injury. Phytother Res 19:796–800

Gurrieri S, Miceli L, Lanza CM, Tomaselli F, Bonomo RP, Rizzarelli E (2000) Chemical characterization of Sicilian prickly pear (*Opuntia ficus* indica) and perspectives for the storage of its juice. J Agric Food Chem 48:5424–5431

Hfaiedh N, Allagui MS, Hfaiedh M, El Feki A, Zourgui L, Croute F (2008) Protective effect of cactus (*Opuntia ficus* indica) cladode extract upon nickel-induced toxicity in rats. Food Chem Toxicol 46:3759–3763

Ikigai H, Nakae T, Hara Y, Shimamura T (1993) Bactericidal catechins damage the lipid bilayer. Biochim Biophys Acta 1147:132–136

Ivanova V, Dörnyei Á, Stefova M, Stafilov T, Vojnoski B, Kilár F, Márk L (2011) Rapid MALDI-TOF-MS detection of anthocyanins in wine and grape using different matrices. Food Anal Meth 4:108–115

Koneman EW (1995) Atlas of Diagnostic Microbiology; Delfino, A, Ed. Italy, pp 549–560

Kulkarni AP, Mahal HS, Kapoor S, Aradhya SM (2007) In vitro studies on the binding, antioxidant, and cytotoxic action of punicalagin. J Agric Food Chem 55:1491–1500

Kuti JO (2004) Antioxidant compounds from four *Opuntia cactus* pear fruit varieties. Food Chem 85:527–533

Lantto TA, Colucci M, Zavadova V, Hiltunen R, Raasmaja A (2009) Cytotoxicity of curcumin, resveratrol and plant extracts from basil, juniper, laurel and parsley in SH-SY5Y and CV1-P cells. Food Chem 117:405–411

Menendez JA, Vazquez A, Colomer R, Brunet J, Carrasco A, Garcia, Fernandez A, Segura A (2007) Olive oil's bitter principle reverses acquired autoresistance to trastuzumab (Herceptin) in HER2-overexpressing breast cancer cells. BMC Cancer 7:80–91

Najjaa H, Zerria K, Fattouch S, Ammar E, Neffati M (2011) Antioxidant and antimicrobial activities of *Allium roseum* L. "Lazoul," a wild edible endemic species in North Africa. Int J Food Prop 14:371–380

Ncibi S, Ben Othman M, Akacha A, Krifi M, Zourgui L (2008) *Opuntia ficus* indica extract protects against chlorpyrifos-induced damage on mice liver. Food Chem Toxicol 46:797–802

Piga A, Del Caro A, Pinna I, Agabbio M (2003) Changes in ascorbic acid, polyphenol content and antioxidant activity in minimally processed cactus pear fruits. LWT-Food Sci Technol 36:257–262

Rodriguez Vaquero MJ, Alberto MR, Manca de Nadra MC (2007) Antibacterial effect of phenolic compounds from different wines. Food Chem 18:93–101

Sanchez-Alonso I, Jimenez-Escrig A, Saura-Calixto F, Borderias AJ (2007) Effect of grape antioxidant dietary fibre on the prevention

of lipid oxidation in minced fish: evaluation by different methodologies. Food Chem 101:372–378

Sogorb MA, Ganzalez GI, Pamies D, Vilanova E (2010) An alternative in vitro method for detecting neuropathic compounds based on acetylcholinesterase inhibition and on inhibition and aging of neuropathy target esterase (NTE). Toxicol in Vitro 24:942–952

Sreekanth D, Arunasree MK, Roy KR, Reddy TC, Reddy GV, Reddanna P (2007) Betanin a betacyanin pigment purified from fruits of *Opuntia ficus*-indica induces apoptosis in human chronic myeloid leukemia Cell line-K562. Phytomedicine 14:739–746

Taguri T, Tanaka T, Kouno I (2004) Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. Biol Pharm Bull 27:1965–1969

Tao Y, Hideaki Y, Toshiro N, Yumi K, Hironori U, Kazuo R (2010) Selective cytotoxicity of glycyrrhetinic acid against tumorigenic r/m HM-SFME-1 cells: potential involvement of H-Ras downregulation. Toxicol Lett 192:425–430

Tesoriere L, Butera D, Pintaudi M, Allegra M, Livrea MA (2004) Supplementation with cactus pear (*Opuntia ficus*-indica)fruit decreases oxidative stress in healthy humans: a comparative study with Vit. C. Am J Clin Nut 80:391–395

Tuberoso CIG, Kowalczyk A, Sarritzu E, Cabras P (2007) Determination of antioxidant compounds and antioxidant activity in commercial oilseeds for food use. Food Chem 103:494–501

Yang SK, Yoneda F, Oide H, Sakae M (1998) Isorhamnetin 3-O-robinobioside, its manufacture, and testosterone 5a-reductase inhibitors and therapeutics containing the flavonoid derivative. Japanese Kokai Tokkyo Koho 7 pp

Zou DM, Brewer M, Garcia F, Feugang JM, Wang J, Zang R, Liu H, Zou C (2005) Cactus pear: a natural product in cancer chemoprevention. Nutrition 4:25–36