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Bioactive Constituents, Antioxidant Activity and in Vitro Cancer Cell Cytotoxicity of Moroccan Prickly Pear (*Opuntia ficus indica* L.) Juices

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Abstract:

Several physico-chemical properties such as fruit pulp weight percentage, acidity, pH, soluble solid content, Antioxidant activity, total phenolic, flavonoid, flavonol and betalain content of nine selected promising cactus pear (*Opuntia ficus indica* L.) accessions were determined. The antioxidant capacity was assessed by means of two different methods: the 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (Trolox equivalent antioxidant capacity) (ABTS) method and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method. The results showed qualitative and quantitative differences in the physico-chemical characteristics of cactus pear accessions. Total phenolics and flavonoids content were found between 354.37 and 643.66 µg gallic acid eq/g and 24.67 and 49.03 µg catechin eq./g dry weight basis. The in vitro cytotoxicity was measured toward P-815 cell line by the growth inhibition assay determined by the MTT viability assay. We found that juice of different cultivars exert a dose-dependent growth inhibition against P-815 cell line. The results provide important information on how to make the best use of cactus pear genotypes investigated for different uses, which is of significance for both technological research and processing practice.

***Keywords**:* prickly pears Juices acidity, sugar content, phenolics, flavonoids, flavonols, betalains, antioxidant activity, anticancer effect

1. Introduction:

Recently, natural foods and food derived antioxidants such as vitamins and phenolic phytochemicals have received growing attention, because they are known to function as chemopreventive agents against oxidative damage and genotoxicity. It is for this reason the consumer demand for fresh ready-to-use products has led, over the last 20 years, to increasing interest in minimally processed fruits and vegetables, as these products combine freshness and convenience (Kim et al 1993).

Medical benefits from plant forms have been recognized for centuries. Herbs have been used in Chinese medicine for thousands of years to cure diseases and heal wounds. Recently, it has been found that components in green tea and grape seeds have anticancer effects (Xu et al. 1992; Kim et al. 2004). Also, as a rule, herbs and natural products lack much of the toxicity that is present in synthetic chemicals, thus, enhancing their appeal for long term preventive strategies.

Opuntia ficus indica, has been used for many years as a common vegetable, is a member of the Cactaceae family, is an important forage crop for livestock in many arid and semi-arid regions of the world. It is widely distributed in Mexico and in all American hemispheres as well as in Africa and in the Mediterranean basin (Acevedo et al. 1985). This plant is pointed out as relevant health promoting food with a great number of potentially active nutrient, the fairly high sugar content and low acidity of the fruit (Sep!ulveda & S!aenz 1990; Joubert 1993) give it a delicious, sweet taste, but at the same time make it very susceptible to microbial invasion, thus limiting its storage life in the fresh state.

Both the cladodes and fruits are frequently consumed both fresh and processed in Latin America (Gurbachan & Felker 1998), whereas only the fresh fruits are more widespread on European and North-American markets

(Butera et al. 2002). Cactus pear is usually consumed fresh, but it is also put to different traditional and industrial uses (Saenz 2000).

Opuntia fruits, also known as cactus pears or prickly pears (figure 1), are regionally consumed as fresh fruit, juice, sweets, etc., but also exported for the European fresh fruit market (Saenz 2000; Feugang et al. 2006).The extracted pigments from prickly pear fruits are used as additives in food, cosmetic, and pharmaceutical preparations (Sep!ulveda & S!aenz 1990; Joubert 1993).

The cactus pear composition and its chemical characteristics were investigated. The composition of cactus pear fruits was studied during the maturation period (El-Gharras et al. 2006), whereas the seeds, skin and pulp were compared in terms of carbohydrate and mineral, composition (El-Kossori et al. 1998). All the authors have agreed that *Opuntia ficus indica* juice was rich in minerals and vitamins (Moßhammer et al. 2006, El-Gharras et al. 2006), and may potentially be included in animal and human diets. Recently, it could be concluded that cactus fruit juice positively affects the body's redox balance, decrease oxidative damage to lipid and improve antioxidant status in diabetic rats (Fatma Hassan Abd El-Razek & Amal Hassan 2001).

In the other hand, fruit and juice of this plant are known by the presence of betalains (betanin and indicaxanthin) (El-Gharras et al. 2008), rarely found in fruits (they occur in red beets), and also elevated in polyphenolic flavonoids (quercetin, kaempferol and isorhamnetin) and various carotenoids, which have antioxidant activity and may protect against human disease (Feugang et al. 2006). These fruits have shown several effect such as antiulcerogenic (Galati et al. 2003), antioxidant (Galati et al. 2003; Kuti 2004; Tesoriere et al. 2004, Dehbi et al. 2013), anticancer (Zou et al. 2005) and hepatoprotective activities (Galati et al. 2005) .

Since high antioxidant capacity is a desired feature for fruits, the aim of this study was to screen the antioxidant capacity, phenolic and betalain composition of nine different types of prickly pears juices and determine their in vitro anticancer effect.

2. Materials and methods

2.1. Raw materials

Nine cultivars of prickly pears fruits *Opuntia ficus indica L* (figure 1), grown in different area in Morocco (table 1), were selected at maximum full maturity without being overripe: yellow species from *Doukkala; Tamellalet; Ras Elain; Ben Guerir; Ait Baamrane; Skhour Rehamna; Alkalaa* and both species red and yellow from *Khouribg*a. For each species, three different lots of fruits were harvested, carefully washed with water to remove the glochids and the obtained juice was centrifuged (4000 x g , 30min at 4°C) and the supernatant juice was stored at -20°C before being used. The seeds were washed abundantly with the water to remove the pulp attached, dried at 60 °C for 24 h. The weight of different components of the prickly pear fruits was determined such as skin, pulp and seeds.

For analysis, triplicate determinations were performed on each sample; data shown later represent the means of three measurements.

Figure 1: Prickly pear (*Opuntia ficus indica* L.)

Table 1. Geographic of provenance of prickly pears fruits used in the study

Area	Latitude N	Longitude W
Khouribga	32°53'	6°54'
Skhour Rehamna	32°29'	7°55'
Alkalaa	32°02'	7°24'
Tamellalet	31°49'	7°30'
Ras Elain	31°48'	7°34'
Ben Guerir	32°14'	7°57'
Doukkala	32°35'	8°39'
Ait Baamrane	29°23'	10°10'

2.2. Experimental material:

The °Brix was determined using a refractometer (ATAGO's Abbe refractometer 1T/4T). The total acidity was determined on 10 ml of juice by measuring the volume of 0.1 N NaOH necessary to take the sample to pH 8.1, which was monitored potentiometrically (Tateo 1978).

The total phenolic contents of prickly pear juice samples were determined using a modified Folin-Ciocalteu method cited by Wolfe et al. (Wolfe et al. 2003). The measurement was expressed as gallic acid equivalents in micrograms per gram of juice.

All determinations of Betalains contents were performed on a UV/Vis spectrometer. The pigments were extracted using the methanol. The betalain content was expressed as mg/L and calculated according to literature (Cai et al. 1998).

The flavonoid content was measured using a colorimetric assay developed by Zhishen et al.(1999). Absorbance was read at 510 nm against the blank (water) and flavonoid content was expressed as µg catechin equivalents/ g DW.

The amounts of flavonols were determined by the method of Yermakov et al. (1987). The same procedure was obtained from a mixture of 2 ml of diluted juice solution, 2ml of aluminium trichloride and sodium acetate. The absorbance at 440nm was recorded. The flavonols content was expressed as rutin equivalents.

The total antioxidant activity of fruit extracts was evaluated using the ABTS radical cation decolorization assay (Miller et al. 1996). ABTS.+ was prepared by reacting ABTS with potassium persulfate (Pellegrini et al. 1999).

The free radical-scavenger activity was determined by the DPPH assay, as described previously by Campos et al. (Campos et al. 2003). Antiradical efficiency was established using regression analysis at a 95% significance level ($P<0.05$). Results are presented in EC50 values, which represent the weight of sample required to scavenge 50% of the DPPH radicals available.

The determination of in vitro cancer cell viability was performed on the cell lines after six passages as previously described by Mossman 1983 and Tilaoui et al. 2011. Briefly, tumor cells were trypsinized, when adherent, (0.15% trypsin, 0.1% EDTA) and 1 to 1.5 x 105 cells/mL were incubated in flat-bottomed 96-well micro titer plates (Bioster, Bastia di Rovolon, Italy) in 100 µL of complete medium. Appropriate dilutions of *Opuntia ficus indica* juices and adriamycin all solubilized in sterilized distillated water were carried out in culture medium before their addition to the cultured cells (final culture volume of 200 µL). After 48 h incubation in humidified atmosphere, at 37 °C and 5% CO2, 20 µL of MTT (5 mg/mL PBS) were added in each well. After 3 h incubation at 37 °C and 5% CO2, 100 µL medium was carefully removed from each well and replaced with 100 µL. Isopropanol-HCl (1:24). After 10 min incubation at 37 °C the solubilized formazan produced by metabolically active cells was measured by scanning the 96-well plates at dual wavelength of 540-630 nm using a Multiskan apparatus (Labsystem, Helsinki, Finland). Using this colorimetric procedure, nine juices and adriamycin, cytotoxic effects could be measured as compared to the viability of untreated cells receiving distillated water alone, according to the following calculation:

$$\% \text{ cell killing} = 100 \times (1 - (ODt / ODo))$$

Where ODo and ODt are the optical density obtained respectively for untreated and juices treated cells. Two independent sets of experiments performed in duplicate were evaluated.

3. Result And discussion

Skhour, Ben Guerir and Khouribga yielded the highest amounts of pulp (Table 2). This yields were probably overestimated since it was difficult to differentiate between peel and pulp tissues. Thus, its pulp and juice probably contained significant amounts of peel components that affected pH and the phytochemical profile. The 59.4and 57.6 % pulp yields obtained from Ben Guerir and red fruit of Khouribga, respectively, were higher and similar compared to yields previously reported by R. A. Chavez-Santoscoy et al. (2009) for opuntia spp. Others authors reported a large variability in pulp yields within the same species ranging from 38 to 62% (Felker et al. 2005). The large differences in yields were related to the prickly pear size, and the amount and thickness of the peels. (Gurrieri et al. 2000) previously documented similar pulp yield for Sicilian cultivars.

The set of prickly pear juices had pH´s ranging from 5.27 to 5.95 (Table 2). These values are less acidic than citrus juices (pH 3.35) (Kelebek et al. 2008). As expected, the juices with the lowest pH values had the highest titritable acidities. Ait Baamrane and Doukkala contained the highest acidities followed by red juice of Khouribga and Ras Elain. Skhour and Ben Guerir contained at least more acidity compared to Tamellalet, yellow juice of Khouribga and Alkalaa. The pH (5.64) and acidity values (0.04%) of Tamellalet are within ranges determined by Gurrieri et al. (2000) in prickly pear juices from Opuntia ficus indica. According to Felker et al. (2005), the pH and acidity for ripen fruits is between 5.6 and 6.5 and 0.05% and 0.18%, respectively. (Viloria-Matos et al. 2002; Díaz-Medina et al. 2007; Pimienta-Barrios et al. 2008) reported more acidic pH in prickly fruits collected from Opuntia dillenni (pH 3.3), Opuntia boldinghii (pH 4.9) and Opuntia joconostle (pH 3.2).

Table 2: Characterization, pulp yield, acidity and sugar contents of juices extracted from nine Moroccan prickly pears.

Cultivars	Fruit characteristics	Pulp Yield %	pH	° Brix	Acidity (%)	°Brix/Acidity
Skhour Rhamna	Spineless, yellow peel, orange yellow pulp.	62,78	5,56±0,07	11,33±0,29	0,067±0,05	169,10±4,34
Alkalaa	Spiny, yellow peel, green-yellow pulp	48,34	5,63±0,05	13,05±0,50	0,049±0,06	266,32±34,94
Yellow Khouribgua	Spiny, green peel, green pulp.	52,00	5,95±0,06	13,76±0,52	0,046±0,01	299,13±10,62
Red Khouribgua	Spiny, purple peel, purple-red pulp.	57,57	5,52±0,09	13,42±1,61	0,077±0,02	174,28±6,80
Tamellalet	Spiny, yellow peel, green-yellow pulp	50,62	5,64±0,27	12,1±1,32	0,046±0,04	263,04±29,07
Doukkala	Spineless, yellow peel, orange yellow pulp.	56,16	5,45±0,16	13,58±0,52	0,081±0,01	167,65±5,31
Ras Eazlain	Spiny, yellow peel, green-yellow pulp.	54,13	5,61±0,11	11,5±2,78	0,074±0,01	155,40±36,15
Ben Guerir	Spiny, yellow peel, green-yellow pulp.	59,41	5,55±0,09	12,17±0,29	0,067±0,06	181,64±3,59
Ait Baamrane	Spineless, yellow peel, orange yellow pulp.	53,20	5,27±0,08	15,47±0,06	0,098±0,05	157,86±0,87

The sugar content of the prickly pear juices varied from 11.33 to 15.47°Brix (Table 2). These values are within the range reported by Mullen et al. (2007) for 13 commercially available fruit juices and drinks of the United Kingdom. The juice with the highest sugar content corresponded to Ait Baamrane with 15.47°Brix. The Ait Baamrane juice also contained more than 15 °Brix. El Garras et al. (2006) reported 16.6 °Brix for a juice extracted from the same species as Ait Baamrane. The prickly pear juice with a high sugar content tend to have lower acidity and therefore a high °Brix/acidity ratio (Table 2)

The total phenolics contents of the juice of the nine cultivars of prickly pears varied from 354.37 to 643.66 µg GAE/g of juice (Table 3). The cultivar from Ait Baamran contained the highest amount of total phenols (643.66 µg GAE/g of juice) followed by Alkalaa cv. (632.11 µg GAE/g of juice). The juice from Khouribga cv. contained the lowest amount. The other cultivars from Skhour Rhamna, Ras Elain, Tamellalet and Ben Guerir contained comparable amounts. The juices of Moroccan origin contained higher phenolic amount than the juices from Mexican prickly pears ranging from 55.4 to 226.3µg GAE/g of juice (Chavez-Santoscoy et al. 2009). But these values are less than those presented by Enza Maria Galati et al. (2003) (746 µg/ml of juice of whole fruits of Sicilian cultivars of prickly pear (Opuntia ficus indica (L.) Mill.) and Chang et al. (2008) (915µg/g GAE in methanol extracts of fruits of opuntia dillenii) The contents of betalains of cultivars (table 3) were similar to

those previously reported by El Gharras et al. (2008); Stintzing et al. (2003); Alfredo Cassano et al. (2010). However, there were wide differences in terms of betaxanthins contents. The cultivars: Ras Elain cv., Alkalaa cv. and Ait Baamrane cv. contained the highest amounts (41.69mg/l - 51.33mg/l) followed by Tamellalet cv. and Skhour Rhamna cv. (36 to 37 mg/l) then Ben Guerir cv., Ben Guerir cv. and Doukkala cv. which contained the lowest amounts (< 23 mg/l). The red juice from Khouribga cv. contained (52.04, 15.84 mg/l) of betacyanins and betaxanthins respectively; on the other hand the yellow juices from the same origin contain only betaxanthins (26.68 mg/l).

Table 3: Total phenols, Flavonoids, Flavonols, Betaxanthins and betacyanins pigments antioxidant activity of juices extracted from nine Moroccan prickly pears.

Cultivars	Total phenols µg GA eq./g of juice	Flavonoids µg catechin eq./g DW	Flavonols µg rutin eq./g DW	Betaxanthins mg indicaxantin/l	Betacyanins mg betanin/l	DPPH EC50 µg/ml of Juice	ABTS mM TE/g DW of Juice
Skhour Rhamna	476,37±5.13	44,56±2.94	23,11±0.62	37,66±4.77	-	91,20±3.63	0.17±0.02
Elkalaa	632,11±5.50	48,42±1.07	14,79±0.76	42,83±3.00	-	65,86±5.20	0.24±0.02
Yellow Khouribga	354,37±5.37	24,67±1.91	18,33±0.99	26,68±2.12	-	135,96±12.50	0.16±0.03
Red Khouribga	358,99±6.10	25,43±1.64	10,7±0.37	15,84±0.08	52,04±0.93	131,82±11.55	0.16±0.03
Tamellalet	467,22±3.79	44,3±1.28	19,04±0.74	36,96±0.34	-	104,71±6.24	0.16±0.02
Doukkala	394,9±7.40	28,35±1.59	19,05±0.67	18,28±1.48	-	112,51±9.83	0.16±0.02
Ras Elain	587,11±4.42	40,26±1.25	19,12±1.26	41,61±2.15	-	75,86±8.16	0.24±0.02
Ben Guerir	524,63±7.27	49,03±3.82	18,81±0.20	22,96±0.24	-	82,86±5.64	0.18±0.02
Ait Baamrane	643,66±3.25	38,76±1.98	32,67±1.93	51,33±4.10	-	52,48±6.17	0.24±0.02

Our results show that the Moroccan juices contained more betaxanthins compared to values found in Mexican prickly pears (Opuntia spp.) (Chavez-Santoscoy et al. 2009) and lower content than those found by Butera et al. (2002) from Italian cultivars of prickly pear (84.20mg per kg of juice).

The effective concentrations (EC50) determined by DPPH radical-scavenging activity for juices varied between 52.48 ± 6.16 and 135.96 ± 12.5 µg/ml of juice (Table 3). The obtained values of DPPH were significantly different according to the varieties of cactus pear juices.

The juices from the nine cultivars were submitted to the ABTS radical cation decolourization assay. All juices had ABTS values in the narrow range of 0.16 to 0.24 mmol TE/g DW (Table 3) despite the significant differences in total phenols and betalains. The Moroccan prickly pear juices ABTS values were higher compared to those (4.20 to 5.31 µmol TE/g of edible pulp) of methanolic extracts from prickly pear fruit reported by Butera et al. (2002) and to values (17.4–25.8 mmol TE/L) obtained by Chavez-Santoscoy et al. (2009).

Flavonoid and flavonol contents in the nine cactus pear fruit juices are shown in (Table 3) were significantly different between the studied Opuntia juice cvs ($p < 0.05$). Flavonoids ranged from 24.67 µg catechin/g in yellow juice from Khouribga to 49.03 µg catechin/g in Ben Guerir cv . Flavonol contents ranged from 10.7 µg rutine /g in red juice from cv Khouribga to 32.67 µg rutin /g in cv Ait Baamrane. Only Ben Guerir cv showed significantly higher flavonoids content than Elkalaa cv. Ait Baamrane, Skhour Rhamna, and Ras Ayn cvs have significantly higher flavonols than red juice from Khouribga cv.

For flavonoid content, values were close to the range reported Kuti et al. (2004) for fruits red-skinned (*O. strepthacantha*) and less than fruits of the green-skinned (*O. ficus-indica*) and the purple skinned (*O. lindheimeri*), wich reported by same authors.

Our results also show that the studied juices contained at least 10 times less flavonoids compared to values found in Mexican prickly pears juice reported by Chavez-Santoscoy et al. (2009) (95.8 - 374.3 µg quercetin eq./g). Ndhlala et al. (2007) analyzed the flavonoids of ethanol extracts of pulp and peels of prickly pears belonging to Opuntia megacantha and found that the pulp contained approximately 10µg/g cathecin. The flavonoid profile has not been reported for the prickly pears analyzed in this study. However it was revealed that the predominant flavonoids in the fruits of Opuntia cactus pears consisted of quercetin, kaempferol and isorhamnetin, respectively. Thus, it is important to continue research on this field to evaluate the flavonoid profile. In addition, The flavonoids constitute about one-half of the 8000 or so recognized phenols and are molecules responsible for the colour of fruit and flowers (Cook & Samman 1996). Thus, the flavonol is one of the most commonly consumed flavonoids and has been well studied for its potential health benefits. Quercetin possesses antiproliferate, anticarcinogenic and antioxidant activities (Kandaswami & Middleton 1994).

The antitumor activity of the products was evaluated against P-815 cell line. The results are summarized in figure 2. It is shown in this figure that the cytotoxic effect depends on the provenance of juice.

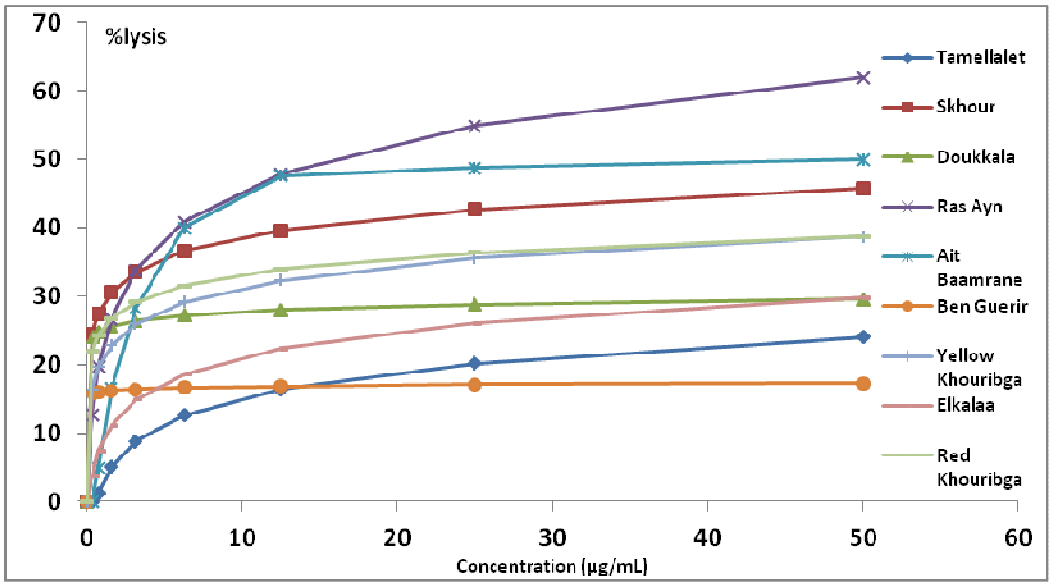

Figure 2: Effect of prickly pear juices on cell viability o four cancer cell line

Overall, the viability of the cancer cells tested was dose-dependent. Furthermore, among the different samples, the juice from Ras Ayn region is the most cytotoxic one and, unlike the other studies juices. Also, Ait Baamrane and Skhour Rhamna juices have an important effect. Interestingly, these cultivars had the highest antioxidant capacity (Table 3). However, comparing the effect of *Opuntia ficus indica* juices on the P815 cell lines, a differential activity can be observed.

Also, it has been reported that the extracts of fruits and stems of cactus exhibit an, anti-tumor activity (Fatma Hassan Abd El-Razek et al. 2012). Furthermore, Chavez-Santoscoy et al. (2009), Reported that the Moradillo juice extracted from Mexican *Opuntia ficus indica* also diminished the growth of normal fibroblasts used as control. The same thing from Rastrero also diminished the growth of prostate cancer cells but did not affect normal fibroblast viability.

Brewer et al. (2005) concluded that Arizona prickly pear cactus effectively inhibited cell growth in several different immortalized and cancer cell cultures in vitro and suppressed tumor growth. The pear extracts significantly suppressed tumor growth in nude mice.

4. Conclusion

From the presented data, it appears that *Opuntia ficus-indica*, has been subject to intensive exploitation due to its great compositional diversity. Nowadays, this hidden knowledge needs to be discovered and re-evaluated. Sophisticated analytical approaches and innovative processing technologies will open new avenues to further promote the use of cactus pear stems, fruits, juices and flowers in food, medicine, cosmetic, and pharmaceutical industries.

This research shows the potential of prickly pears as an important source of natural antioxidants and nutraceuticals. Further research is needed in order to find the most bioactive anticancer compounds and if the in vitro results correlate with animal studies.

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