academicJournals

Vol. 12(40), pp. 5875-5885, 2 October, 2013
DOI: 10.5897/AJB12.612
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Upshot of the ripening time on biological activities, phenol content and fatty acid composition of Tunisian *Opuntia ficus-indica* fruit

Amel Rabhi[1]*, Hanen Falleh[2], Ferid Limam[3], Riadh Ksouri[2], Chedly Abdelly[2] and Aly Raies[1]

[1]Laboratoire de Microorganismes et Biomolécules Actives, Faculté des Sciences de Tunis (FST), Campus Universitaire 2092 El-Manar II, Tunis- Tunisia.
[2]Laboratoire des plantes extrêmophiles, Centre de Biotechnologie à la Technopole de Borj-Cédria (CBBC), BP 901, 2050 Hammam-lif, Tunisia.
[3]Laboratoire des Substances Bioactives, Centre de Biotechnologie à la Technopole de Borj-Cédria (CBBC), BP 901, 2050 Hammam-lif, Tunisia.

Accepted 9 January, 2013

Total phenol content and the antioxidant activities of three cultivars of *Opuntia ficus-indica* (L.) Mill. were evaluated. The results show that the ecotypes were significantly different according to their fatty acid composition, antioxidant and antibacterial activity, as well as their polyphenol profiles. Rossa fruit collected in August exhibited the higher phenolic content (15.48 mg GAE g^{-1} DW) with a stronger antioxidant activity. Bianca fruit collected in August exhibited a lower phenolic content (3.13 mg GAE g^{-1} DW) together with the stronger antiradical activity (96.14%). The advantage of this ecotype was more discernible as compared to positive controls butylated hydroxytoluene (BHT, 79.75%). Likewise, CpG chromatogram identification revealed also an important difference between the ecotypes on oleic and linoleic acids.

Key words: *Opuntia ficus-indica*, variability, phenol content, fatty acid, antioxidant activity.

INTRODUCTION

Opuntia ficus-indica are an important source of alimentary nutrients and vitamins (Sawaya et al., 1983; Teles et al., 1984). The fruits are eaten fresh, dried or preserved in jams, syrups or processed into candy-like products (Hoffman, 1980). Their juices are sometimes fermented, either into ethanol or wine and other beverages or used in food flavourings and colourings (Bustos, 1981; Retamal et al., 1987; Saenz, 1996; Gurrieri et al., 2000). Cactus pear could have a double application, both becoming an option for obtaining natural colouring features and providing health benefits through its antioxidant function (Stintzing and Carle, 2004; Tesoriere et al., 2005). It has been reported that the extracts of fruits and stems exhibits hypoglycemic (Ibañez-Camacho and Roman-Ramos, 1979; Trejo-Gonzalez et al., 1996) anti-ulcer, (Galati et al., 2001; Lee, 2002), and anti-allergic actions (Lee et al. (2000). In addition, Park et al. (1998) reported analgesic and anti-inflammatory actions of the fruit and stem extracts. Recently, the methanol extract of *O. ficus-indica* fruits was shown to inhibit free radical-induced neuronal injury in mouse cortical cultures (Wie, 2000).

Furthermore, it was shown that cactus pear seed is rich in oleic acid (C18:1) and linoleic (C18:2) acids (16.7 and

*Corresponding author. E-mail: espoirsr@yahoo.fr. Tel: +216 93218013.

Abbreviations: BHT, Butylated hydroxytoluene; **BHA,** butylated hydroxyanisole; **RFA,** Rossa Fruit of August; **GFA, G**ialla Fruit of August; **BFA,** Bianca Fruit of August; **RFN,** Rossa Fruit of November; **GFN,** Gialla Fruit of November; **BFN,** Bianca Fruit of November.

70.3%, respectively), which representing 87% of the total fatty acids (Ennouri et al., 2005). At the beginning of the 19th century, commercial populations were established on the island of Sicily to meet the increasing demand for late-ripening fruits, produced through "scozzolatura". This technique consists of removing the flowers and cladodes spring flush to get a second bloom, causing the fruits to ripen in October to November, 2 to 3 months later than the summer crop (Barbera 1992; 1993). It has been demonstrated that the great biodiversity in fruit colours (red, orange, purple, pink, yellow, and lime-green), results in great potential for the flat stemmed *Opuntias* in arid lands (Griffiths and Hare, 1907; Pimienta, 1990; Barbera et al., 1995; Inglese et al., 1995; Parish and Felker, 1997). Cultivars for fruit production can be distinguished by the colour of the fruit, peel and the ripe flesh, which can be red-purple, yellow-orange, white-cream, or greenish-white flesh. Cultivars also differ in plant shape, vigour, fertility, cladode and fruit size, fruit ripening time, seed count and ability to reflow (Wessels, 1988; Pimienta, 1990; Barbera, 1993). Interestingly, some works reported the presence of phenolic compounds in fruit samples of cactus pears (Kuti, 2000; Lee and Lim, 2000; Morales et al., 2007). Polyphenols constitutes the main powerful compound, on account of their multiple applications in food industry, cosmetic, pharmaceutical and medicinal materials (Maisuthisakul et al., 2007).

Structurally, phenolics embrace an aromatic ring, bearing one or more hydroxyl substituent, and range from simple phenolic molecule to highly polymerised compounds (Bravo, 1998). In addition to their role as antioxidant, these compounds exhibit a wide spectrum of medicinal properties, such as anti-allergic, anti-arthero-genic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio-protective and vasodilatory effects (Balasundram et al., 2006). The literature reports few scientific studies regarding the presence of phenols or other antioxidant compounds in cactus pear fruits. In fact, the purple cultivar has the highest concentration of total phenols, at approximately 660 mg/l juice (Stintzing et al., 2005). Other studies have identified the presence of flavonoids as flavonol glycosides, amongst which significant amounts of isorhamnetin-3- rutinoside, rutin and kaempferol-3-rutinoside were found in a blend of yellow and red cultivars (Galati et al., 2003). Besides, according to Wallace (1986), flavonoids content in *Opuntia* fruits vary with variety, tissue type, and maturation. Subsequently, previous studies have shown that plant polyphenolics, antioxidant and antimicrobial activities depend significantly on biological factors (genotype, organ and ontogeny), as well as, edaphic and environmental (temperature, salinity, water stress and light intensity) ones (Lisiewska et al., 2006).

The objectives of this study were to investigate antioxidant activities using different tests, to estimate the antimicrobial capacities against human pathogen strains that may be from food poisoning microorganisms, and to quantify and identify the fatty acid present in *O. ficus-indica* fruits.

MATERIALS AND METHODS

Chemical and reagents

Folin-Ciocalteu reagent, butylated hydroxytoluene (BHT), nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), vanillin, nicotinamid-adenin-dinucleotid (NADH), 1,1- diphenyl-2-picrylhydrazyl (DPPH), sodium bicarbonate ($NaCO_3$), and nitrite sodium ($NaNO_2$) were purchased from Sigma-Aldrich (GmbH, Sternheim, Germany). Authentic standards of phenolic compounds (gallic acid and catechin) were purchased from Sigma and Fluka (Buchs, Switzerland).

Plant sampling

Three *O. ficus-indica* cultivars of different colours (Rossa, Gialla, Bianca) were used in this study (Photo 1). Fruits were harvested in August [Rossa Fruit of August (RFA), Gialla Fruit of August (GFA) and Bianca Fruit of August (BFA)], and in November [(Rossa Fruit of November (RFN), Gialla Fruit of November (GFN) and Bianca Fruit of November (BFN)] in the same year (2009) when "scozzolatura" is applied at full ripening in Kasserine locality (286 km center-west Tunis; semi arid bioclimatic stage; mean annual rainfall: 335 mm/year). The samples were rinsed with distilled water then freeze-dried and ground to a fine powder and stored at - 20°C until analysis began.

Extraction of total lipid because soxhlet method is more efficient

To determine de oil content the ISO 659-1988 (E) procedure was used. Soxhlet extractions were performed using 40 g of cactus. The amount was transferred in a 33×100 cellulose thimble and placed in the extraction chamber of a 200 ml capacity Soxhlet apparatus. The cellulose thimble was plugged with cotton in order to avoid transfer of sample particles in the distillation flask fitted with a condenser, placed on a 500 ml distillation flask containing 300 ml of solvent and 3 boiling glass regulator. Samples were thus, extracted under reflux with *n*-hexane during 4 h (18 to 22 cycles/h). Thereafter, the cellulose cartridge was cooled to room temperature in a desiccators and its content was then milled before being transferred again in the thimble. The described procedure was thus repeated within 2 h until a total extraction of 8 h (4 + 2 + 2 h). After the extraction, the major solvent was eliminated in a vacuum rotary evaporator. The content was then transferred in a smaller tarred flask and concentrated to dryness with a vacuum rotary evaporator during 1 h at 80°C before cooling again in desiccators for 1 h. The flask was then weighed and the operation repeated during 30 min until difference between two consecutive weights was less than 10% (m/m). Extractions were performed at least three times and the mean values were reported. Results obtained were expressed as described hereinafter:

$$\% \text{ Oil content} = \frac{\text{Weight of oil obtained after extraction}}{\text{Weight of dry sample}} \times 100$$

Analysis of fatty acid composition

Fatty acid methyl esters (FAMEs) of the studied oil samples were

prepared based on a method described by Parish and Felker (1997). Briefly, 0.1 g of oil was weighed in a 20 ml test tube (with screw cap). The sample was dissolved in 3 ml of hexane, and 0.5 ml of potassium hydroxide (2N) in methanol was added. The test tube was capped and vortexes for 30 s. The mixture was centrifuged at 402 × *g* for 10 min, and the extracts was transferred to a 2 ml auto sampler vial, and analyzed using gas chromatography.

Gas chromatography (GC) condition

Fatty acid composition of oil samples were analyzed using a GC system (Agilent 6890, Wilmington, Delware, USA) equipped with a split-splitness injector. Hewlett-Packard EL-980 flame ionization detection (FID) system was used to separate and quantify each FAMEs component. FAMEs were separated using DB-23 column (30 m × 0.25 mm, i.d. 0.25 µm polyethylene glycol film (Muskegon, Michigan, USA). Chromatography data was recorded and integrated using Chemstation software (version 6.0, Hewlett-Packard, Waldbronn, Germany). Oven temperature was held at 50°C for 1 min, then increased to 175°C at 4°C/min and increased to 230°C, held for 5 min at 230°C. The temperatures of injector and detector were set at 250 and 280°C. Oil sample (1 µL) was injected with split ratio of 1:50 at column temperature of 110°C. Helium (1 ml/min) was used as carrier gas controlled at 103.4 kPa, while hydrogen and air were used for FID and was held at 275.6 kPa. FAME standards were used to determine each type of fatty acid. Identification of fatty acids of the samples was carried out by comparing the retention times of reference standards and was analyzed under the same operating conditions as those employed for FAME of the standards and was expressed in percentage.

Determination of antioxidant activities and polyphenolic contents

Extraction of phenolic compounds

Sample extracts were obtained by magnetic stirring of 2.5 g of dry fruits powder with 25 ml of pure methanol for 30 min (Mau et al., 2005). All extracts were kept for 24 h at 4°C, filtered through a Whattman No. 4 filter paper, and evaporated under vacuum to dryness. They were stored at 4°C until analysis began.

DPPH radical-scavenging activity

The DPPH quenching ability of plant extracts was measured according to Hanato et al. (1988). One ml of the extract at different concentrations was added to 0.25 ml of a DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm. The ability to scavenge the DPPH radical was calculated using the following equation**:**

DPPH scavenging effect $AA\ (\%) = [(A_0 - A_1)/A_0] \times 100$ (1)

Where, A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min. All samples were analyzed in three replications. A higher percentage value corresponds to a higher antiradical activity of plant extract.

Iron reducing power

The capacity of plant extracts to reduce Fe^{3+} was assessed by the method of Oyaizu (1986). Methanol extract (1 ml) was mixed with 2.5 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide, and the mixture was incubated at 50°C for 20 min. After that, 2.5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged at 650 × g for 10 min. The upper layer fraction (2.5 ml) was mixed with 2.5 ml of deionised water and 0.5 ml of ferric chloride and thoroughly mixed. The absorbance was measured at 700 nm and ascorbic acid was used as a positive control. A higher absorbance indicates a higher reducing power. The results are expressed in µg/ml values.

***β*- Carotene bleaching test (BCBT)**

A modification of the method described by Koleva et al. (2002) was employed. *β*- Carotene (2 mg) was dissolved in 20 ml chloroform. To 4 ml of this solution, linoleic acid (40 mg) and Tween 40 (400 mg) were added. Chloroform was evaporated under vacuum at 40°C and 100 ml of oxygenated ultra-pure water was added, then the emulsion was vigorously shaken. An aliquot (150 µL) of the *β*-carotene: linoleic acid emulsion was distributed in each of the wells of 96-well micro titre plates and fruits methanolic extracts (10 µL) were added. Three replicates were prepared for each of the samples. The micro titre plates were incubated at 50°C for 120 min, and the absorbance was measured using a model EAR 400 micro titre reader (Labsystems Multiskan MS) at 470 nm. Readings of all samples were performed immediately (t = 0 min) and after 120 min of incubation. The antioxidant activity (AA) of the extracts was evaluated in term of *β*-carotene blanching using the following formula:

$$AA\ (\%) = [(A_1 - A_0)/(A_0 - A_1)] \times 100 \quad (2)$$

Where, A_0 is the absorbance of the sample at 0 min, and A_1 is the absorbance of the sample at 120 min. The results are expressed in percentage.

Screening for antimicrobial activity

The antibacterial activity of fruit extracts was assessed by the agar disk diffusion assay (Mann, 2004) against four human pathogenic bacteria: Gram-positive cocci including *Staphylococcus aureus* (ATCC 25923) and Gram-negative bacteria including *Escherichia coli* (ATCC 35218), *Enterococcus facium*, and *Salmonelle typhi.* The bacterial strains were first grown on Muller Hinton medium at 37°C for 24 h prior to seeding onto the nutrient agar. One or several colonies of the indicator bacteria were transferred into API suspension medium (Bio Mérieux) and adjusted to the 0.5 McFarland turbidity standard with a Dens mat (Bio Mérieux). A sterile filter disc with 6 mm in diameter (What man paper no. 3) was placed on the infusion agar seeded with bacteria, and 10 µL of several extract concentrations were dropped onto each paper disc, representing. The treated Petri dishes were kept at 4°C for 1 h, and incubated at 37°C for 24 h. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the discs. Standard discs of gentamycin (10 UI) served as positive antibiotic controls according to CASFM 2005 guidelines.

Discs with 10 µL of pure methanol were used as negative controls. For the antifungal activity, the agar-disc diffusion method was used as previously described (Cox et al., 2000). One Candida strains (*Candida albicans*) was first grown on Sabouraud chloramphenicol agar plate at 30°C for 18 to 24 h. Several colonies were transferred into API suspension medium and adjusted to two McFarland turbidity standards. The Inoculate of the respective yeasts were streaked onto Sabouraud chloramphenicol agar plates at 30°C using a sterile swab and then dried. A sterile filter disc, diameter 6 mm (What man paper no. 3) was placed in the plate. Ten micro litres of extract concentration were dropped on each paper disc. The treated Petri dishes were placed at 4°C for 1 to 2 h

Table 1. Levels and fatty acid compositions of total lipids (TL) in *Opuntia ficus-indica* fruit.

Fatty acid	Relative content (%)[a]				
	RFA	BFA	GFA	GFN	RFN
C12:0	0.01±0.00	0.01±0.00	0.07±0.07	0.08±0.00	0.1±0.00
C14:0	0.12±0.00	0.11±0.01	0.15±0.08	11.47±0.24	0.09±0.01
C16:0	12.48±0.05	12.15±0.5	12.04±0.98	1.24±0.50	11.44±0.24
C16:1	1.36±0.00	1.25±0.07	1.05±0.79	1.82±0.23	0.69±0.1
C17:0	8.32±0.00	NI	5.18±5.75	NI	NI
C18:0	1.73±0.00	1.76±0.04	2.55±0.21	19.32±0.28	1.96±0.04
C18:1	18.88±0.10	24.59±0.08	16.90±1.29	63.57±0.19	18.46±0.19
C18:2	55.40±0.05	58.41±0.27	59.05±5.39	0.94±0.02	64.60±0.12
C18:3	0.88±0.00	0.54±0.00	1.69±0.97	0.22±0.03	1.34±0.00
C20:0	0.16±0.00	0.27±0.00	0.28±0.06	0.33±0.00	0.24±0.00
C20:1	0.23±0.00	0.38±0.01	0.44±0.09	0.20±0.02	0.44±0.1
C22:0	NI	0.19±0.01	0.37±0.07	0.28±0.03	0.30±0.08
C22:1	NI	0.31±0.01	0.16±0.08	0.48±0.01	0.38±0.03
U/S[b]	3.35±0.05	5.89±0.47	3.83±6.68	2.05±0.51	6.11±0.14

[a]Results are given as the average of triplicate determinations standard deviation, [b]Ratio of unsaturated to saturated fatty acids. NI, Not identified. **RFA,** Rossa Fruit of August; **GFA, G**ialla Fruit of August; **BFA,** Bianca Fruit of August; **RFN,** Rossa Fruit of November; **GFN,** Gialla Fruit of November; **BFN,** Bianca Fruit of November.

and then incubated at 37°C for 18 to 24 h. As for the antibacterial activity, the antifungal one was evaluated by measuring the diameter of the growth inhibition zone around the discs. The susceptibility of the standard was determined using a disc paper containing 20 µg of amphoterecin B. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded.

Colorimetric quantification of phenolics

Determination of total polyphenol content

An aliquot of 125 µL of diluted extract were added to 500 µL of distilled water and 125 µL of the Folin-Ciocalteu reagent. The mixture was shaken, before adding 1250 µL Na_2CO_3 (7%), adjusting with distilled water to a final volume of 3 mL, and mixed thoroughly. After incubation for 90 min at 23°C in the dark, the absorbance versus prepared blank was read at 760 nm (Dewanto et al., 2002). Total phenol content of fruits was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid, ranging from 0 to 400 µg/mL. All samples were analysed in triplicate.

Estimation of total flavonoid content

Total flavonoids were measured by a colorimetric assay (Dewanto et al., 2002). An aliquot of diluted sample or standard solution of (+)-catechin was added to a 75 µL of $NaNO_2$ solution (5%), and mixed for 6 min before adding 0.15 ml $AlCl_3$ (10%). After 5 min, 0.5 ml of NaOH was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the blank where the sample was omitted. Total flavonoid content was expressed as mg catechin per gram of DW (mg CE/g DW), through the calibration curve of (+)-catechin, ranging from 0 to 400 µg/mL. All samples were analysed in triplicate.

Quantification of total condensed tannins

To 50 µL of properly diluted sample, 3 ml of 4% vanillin solution in methanol and 1.5 ml of concentrated hydrochloric acid were added (Sun et al., 1998). The mixture was allowed to stand for 15 min, and the absorption was measured at 500 nm against methanol as a blank. The amount of total condensed tannins is expressed as mg (+)-catechin/g DW. The calibration curve range of catechin was established between 0 and 400 µg/ml. All samples were analysed in triplicate.

Statistical analysis

For all cultivars parameters, three replicates were used. To determine their relative variability on phenol content, fatty acid composition, antioxidant and antibacterial activities. Analysis of variance (ANOVA) was achieved for whole data, using the XLSTAT statistical program. Means were compared using the Newman-Keuls (SNK) test at the $P<0.05$ level, when significant differences were found.

RESULTS

Fatty acid compositions

In our study, the fatty acid composition differed greatly between *O. ficus-indica* fruit depending on pulp colour and ripening time. Typical CpG chromatogram of fruit extracts is presented in Table 1. Thirteen (13) fatty acids were identified in fruit (pulp and seed) extracts. Major fatty acids were significantly different when comparing the ecotypes. Wherein the analysis of FAME exhibited that the major fatty acids in summer crop are linoleic C18:2 (Ω6) (55.40; 58.41 and 59.05 %) followed by oleic C18:1 (18.88; 24.59 and 16.90%) and palmitic acids C16:0

(12.48; 12.15 and 12.0 4%) for RFA; BFA and GFA, respectively. The fatty acid composition of RFN presented nearly the similar trends to that of summer crop with linoleic C18:2 (Ω6) (64.60%) followed by oleic C18:1 (18.46%) and palmitic C16:0 (11.44%) acids. Whereas the major fatty acids in GFN are oleic C18:1 (63.57%) followed by stearic C18:0 (19.32%) and myristic C14:0 (11.44%) acids. Results analysis illustrate that "scozolatturae" technique probably had an effect on linoleic and myristic acids composition of Gialla fruit collected in November as compared to those harvested in August.

Antioxidant activities

Antiradical activity

DPPH is a free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). The reduction capability of DPPH radical was determined by the decrease in absorbance induced by plant antioxidants. BHT was the reagent used as standard. The scavenging effect of methanol extracts and standard on the DPPH radical expressed in percentage was in the following order: Bianca fruit collected in August (96.14%) > Gialla fruit collected in August (93.15%) > Rossa fruit collected in August (93.15%) Rossa fruit collected in November (85.51%) Bianca fruit collected in November (39.65%) Gialla fruit collected in November (32.37%) (Figure 1A). The experimental data of this species reveals that ecotype extracts have a stronger effect of scavenging free radical than positive control BHT (79.75%).

Iron reducing power

Another reaction pathway in electron donation is the reduction of an oxidized antioxidant molecule to regenerate the ‘‘active” reduced antioxidant. Iron reducing power values of all tested samples were ranged from 0.04 to 0.55 µg mL^{-1} and arranged in the following increasing efficiency order: ascorbic acid (1.32 µg ml^{-1}) > Bianca fruit (August) (0.55 µg ml^{-1}) > Gialla fruit (August) (0.45 µg ml^{-1}) > Rossa fruit (August) (0.16 µg ml^{-1}) > Rossa fruit (November) (0.10 µg ml^{-1}) > Bianca fruit (November) (0.05 µg ml^{-1}) > Gialla fruit (November) (0.04 µg ml^{-1}). As shown in (Figure 1B), the reducing power of fruits collected in August extracts was clearly more important than fruits collected in November extracts. Nonetheless, the ascorbic acid (positive control) concentration required to reduce the ferric iron was higher (1.32 µg/ ml) than all extracts, indicating probably a low activity.

Antioxidant assay using *β*-carotene linoleate system

In this model, β-carotene undergoes rapid discoloration in the absence of an antioxidant. The presence of an antioxidant such as phenolics can hinder the extent of β-carotene destruction by ‘‘neutralizing” the linoleate free radical and any other free radicals formed within the system (Kamath and Rajini, 2007). Figure 1C depicts the inhibition of β-carotene bleaching by the fruit extracts of *O. ficus-indica*, and by the two positive controls [butylated hydroxyanisole (BHA) and BHT].

In terms of β-carotene bleaching effect, those samples exhibited the following order: BHT (79.75 %) > BHA (61.69 %) > GFN (26.16 %) > BFN (18.33 %) > RFN (15.36 %) > GFA (8.37 %) BFA (7.57 %) > RFA (3.39 %). This method showed significant differences between the different ecotypes. Indeed, fruits collected in November were statistically as efficient as those collected in August.

Antimicrobial activity

Table 2 shows the antibacterial activities of *O. ficus-indica* fruits measured by the agar diffusion method against selected pathogenic bacteria. The mean inhibition zone for all bacteria treated with Rossa fruit extracts varied from 9.33 to 12 mm and from 7.33 to 11 mm at 50 mg/l treated with Bianca fruit varied from 8.33 to 10.33 mm and from 7.33 to 10 mm and treated with Gialla fruit varied from 6.33 to 8mm and from 5.33 to 7 mm, respectively for ecotype collected in November and ecotype collected in August.

The strongest activity was recorded against *Enterococcus facium* for the two ecotypes with an inhibition zone equal to 12 and 11 mm, respectively for RFN and RFA. Pertaining to antifungal tests, fruit extracts concentration (50 mg/ml) inhibit *Candida albicans* growth with an inhibition zone equal to 9.33 and 7.33 mm, respectively for RFN and RFA. These results suggest in one hand that “scozolatturae technique” had an impact on antibacterial activities of *O. ficus-indica* fruit, in the other hand that methanolic extracts of fruit were more efficient to inhibit bacterial growth than fungal one for the two ecotypes, probably in relation to their active molecules.

Analysis and quantification of phenolic compounds

Total phenolics contents in Opuntia ficus-indica fruit

Results of total phenolic, flavonoid, and condensed tannin quantifications are represented in Figure 2. Total phenolic content measured on *O. ficus-indica* fruit varied significantly depending on ripening-time and on ecotypes and ranged from 2.61 to 15.48 mg GAE g^{-1} DW. Among ecotypes, RFA was the richest in phenolics.

They were significantly decreased in the order: RFA (15.48 mg GAE g^{-1} DW) > GFA (7.31 mg GAE g^{-1} DW) > GFN (3.89 mg GAE g^{-1} DW) > BFA (3.13 mg GAE g^{-1} DW) > BFN (2.90 mg GAE g^{-1} DW) > RFN (2.61 mg GAE g^{-1} DW).

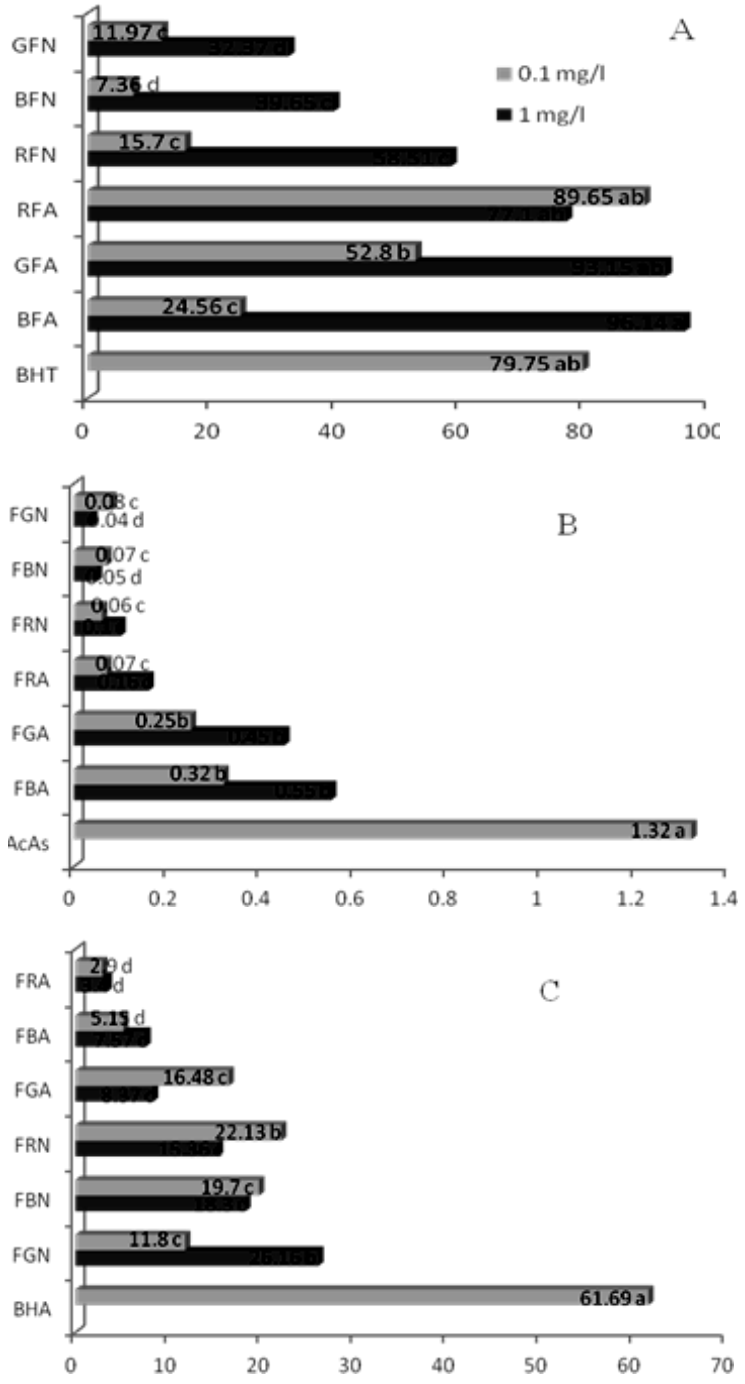

Figure 1. 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (A), reducing power (B) and β-carotene bleaching inhibition capacity (C) in the *Opuntia ficus-indica* fruit and authentic standards (BHT, ascorbic acid and BHA). Means of three replicates followed by the same letter are not significantly different at $P < 0.05$ according to the Newman-Keuls post-hoc test.

Total flavonoid content of Opuntia ficus-indica extracts

Variability of total flavonoid contents presented similar trends to that of total polyphenols. Thus, Fruits collected in August had the highest total flavonoids. Total flavonoid values were significantly decreased in the order: GFA (5.43 mg CE g^{-1} DW) > RFA (3.79 mg CE g^{-1} DW) > BFA

Table 2. Antimicrobial activity of *Opuntia ficus-indica* fruit extracts against five human pathogen bacteria, compared to that of positive standard (gentamycin) at 50 mg/ml. Inhibition zone calculated in diameter around the disc (mm ± SD).

Bacterial strain	Diameter of growth inhibition (mm ± SD)[a]						
	Gentamycin	RFN	RFA	BFN	BFA	GFN	GFA
Echerchia coli ATCC 35218	22.23±0.67	11.66 ± 1.06	7.66 ± 1.40	11.66 ± 1.06	10.33 ± 2.06	6.33 ± 1.06	6.66 ± 2.06
Staphylococcus aureus ATCC 25923	18.3±0.6	9.66 ± 2.32	10 ± 1.84	11.66 ± 1.06	10.33± 1.06	8.00 ± 1.66	7.33 ± 2.46
Salmonella thyphi	24.7±0.60	10 ± 0.92	9 ± 0.92	11.66 ± 1.06	9.66 ± 1.86	7.33 ± 1.96	5.33 ± 1.96
Enterococcus facium	22.95 ±0.66	12 ± 0.92	11 ± 0.92	11.66 ± 1.06	8.33 ± 1.46	7.66 ± 1.86	7.00 ± 1.99
Candida albicans	19.85±0.66	9.33 ± 2.32	7.33 ± 1.40	11.66 ± 1.06	9.66 ± 1.06	6.66 ± 1.06	6.66 ± 1.89

[a]Data are reported as means ± SD of three measurement. The diameter of disc was 6 mm. SD, Standard deviation.
RFA, Rossa Fruit of August; **GFA, G**ialla Fruit of August; **BFA,** Bianca Fruit of August; **RFN,** Rossa Fruit of November; **GFN,** Gialla Fruit of November; **BFN,** Bianca Fruit of November.

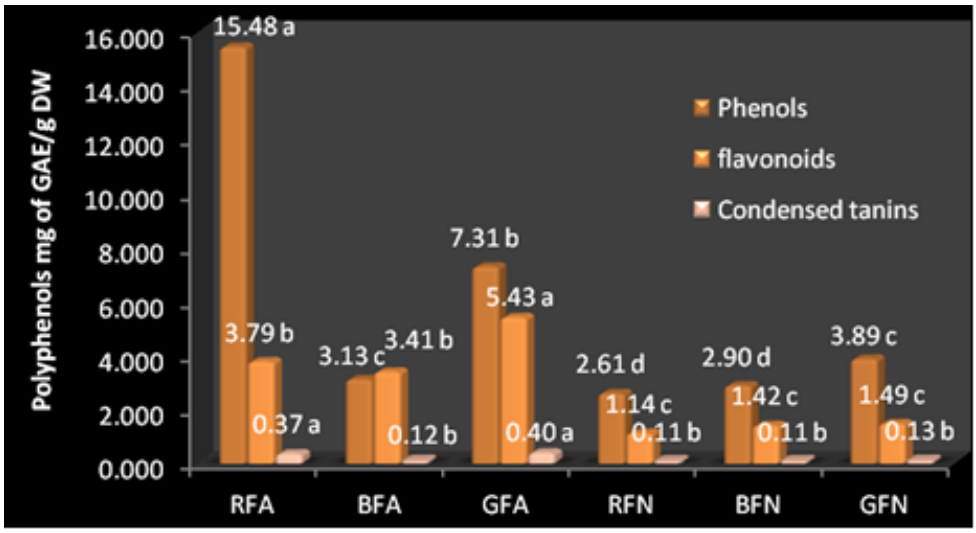

Figure 2. Total polyphenol, flavonoid, and condensed tannin of *Opuntia ficus-indica* cultivars. Means (three replicates) followed by the same letter are not significantly different at P < 0.05 according to the Newman-Keuls post-hoc test. **RFA,** Rossa Fruit of August; **GFA, G**ialla Fruit of August; **BFA,** Bianca Fruit of August; **RFN,** Rossa Fruit of November; **GFN,** Gialla Fruit of November; **BFN,** Bianca Fruit of November.

(3.41 mg CE g^{-1} DW) > GFN (1.49 mg CE g^{-1} DW) > BFN (1.42 mg CE g^{-1} DW) > RFN (1.14 mg CE g^{-1} DW).

Condensed tannins in methanolic extract

Condensed tannin content measured varied significantly, depending on ripening-time and on ecotypes and ranged from 0.40 to 0.11 mg CE g^{-1} DW. Hence, Gialla and Rossa fruit collected in August displayed the highest values (0.40 and 0. 37 mg CE g^{-1} DW, respectively) as compared to Bianca fruit collected in August (0.11 mg CE g^{-1} DW).

DISCUSSION

For a plant to be suitable for oil production, it must meet the following two criteria:

1. The oil content must reach the minimum for commercially viable exploitation, and

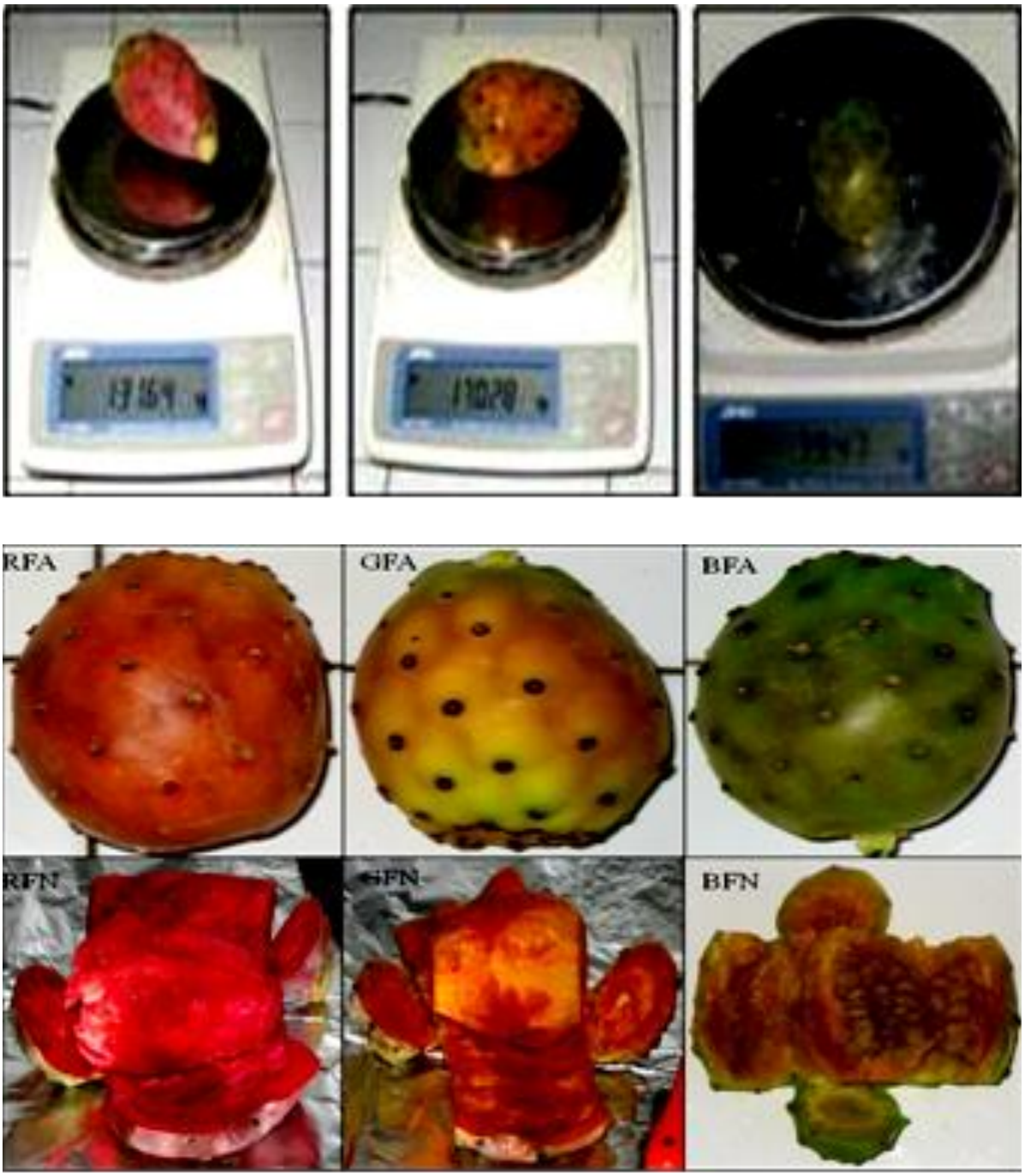

Photo 1. *Opuntia Ficus-Indica* fruits. **RFA,** Rossa Fruit of August; **GFA, G**ialla Fruit of August; **BFA,** Bianca Fruit of August; **RFN,** Rossa Fruit of November; **GFN,** Gialla Fruit of November; **BFN,** Bianca Fruit of November.

2. The plant must be suitable for high acreage cultivation.

The only exceptions are plants that contain oils or fats unique in their composition or with properties that cannot be found elsewhere (Bockisch, 1998). Cactus pear pulp which resembles the edible part of the fruit can be divided into seeds (15%) and strained pulp (85%), the latter being the basis for fruit and juice products. It is find that seeds contain the maximum amount of oil (98.8 g/kg dry weight) while total lipid, recovered from lyophilised strained pulp, accounted for 8.70 g/kg. It is well known that the mesocarp, or pulp, of fruits generally contains very low levels of lipid materials (0.1-1.0%) and, as such, does not constitute an important source of edible or industrial oils (Kamel and Kakuda, 2000). Amounts of oil recovered from seeds which represent a potential source

of oil, are in agreement with literature data (Pimienta-Barrios, 1994; Sawaya and Kahn, 1982). The levels of total lipids, however, may depend on fruit cultivar, degree of ripeness and fruit processing or storage conditions. Previous data on cactus pear seed oil show rather, a similar pattern in that linoleic acid is the fatty acid marker (Sawaya and Kahn, 1982). Fatty acid profile of seed oil evinces the lipids as a good source of the nutritionally essential linoleic acid and unsaturated oleic acid, wherein the ratio of linoleic acid to oleic acid was about 3:1. In both seed and pulp oils, linoleic acid is the dominating fatty acid, followed by palmitic and oleic acids, respectively (Ramdan, 2003). Based on our results, high percentage of palmitic acid (C16:0) in Rossa fruit oil can be exploited as new source of vegetable oil. Additionally, the high palmitic and linoleic acids content will be advantageous in hyperchlosterolicmic conditions (Kuti, 1992; 2004).

Additionally, our findings show that *Opuntia* oil may possess comparable nutritional content, as the palm oil, which has been extensively accepted and used as cooking oil in various food applications; due to its superior frying quality and oxidative stability owing to high content of monounsaturated and saturated fatty acids (Saenz et al., 1993). As recommended by the National Cholesterol Education Program/American Heart Association, C16:0 and C18:0 are best saturated fatty acid (SFA) from natural source (Odoux and Dominguez, 1996). It is found that antioxidant molecules such as ascorbic acid, tocopherol, flavonoids, and tannins reduce and decolourize DPPH due to their hydrogen donating ability (Kumaran and Karunakaran, 2007). Phenolic compounds of the *O. ficus-indica* fruit extracts were probably involved in their antiradical activity. Several studies attributed the inhibitory effect of plant extracts against bacterial pathogens to their phenolic composition (Kamath and Rajini, 2007; Espinet al., 2007). The inhibitory effect of these phenolics could be explained by adsorption to cell membranes, interaction with enzymes, substrate and metal ion deprivation (Naczk and Shahidi, 2006).

Our results suggest that the antibacterial capacity needed, as compared to antioxidant activity, which has a good efficiency with crude extracts of *O. ficus-indica* fruit, more concentration and even purification of phenolic compounds. In fact, numerous studies evocate the analgesic and anti-inflammatory actions of the genus *Opuntia* by using the phytosterols from fruit and stem extract (Parck et al., 1998). Gastric lesions in rat animal studies were reduced both by stem and fruit powder (Lee et al., 2001; 2002). Besides, the ethanol extract of *O. ficus-indica* fruits inhibited the writhing syndrome induced by acetic acid, indicating that, they contain analgesic effect (Parck et al., 1998). Hence, our findings shows that ripening time, climatic conditions and cultivars had significantly and differently affect phenol content of *O. ficus-indica* fruits compared with those harvested in August in Morocco by Maataoui et al. 2006 (0.2 and 0.28 mg/100 ml for Gialla and purple, respectively). Moreover, total phenolic content of RFA was higher than that reported by Stintzing et al. (2005) of 660 mg/l, by Morales (2007) of 777.4 mg/l and by (Maataoui et al., 2006) of 0.2 and 0.28 mg/100 ml for Gialla and Rossa fruit, respectively. Results depicted also, that summer crop displayed much higher polyphenol content than those collected in November. Probably, this can be attributed to the “scozolattura” technique, which had a positive impact on fruit size other than seems to exert a negative effect on phenol content.

Accordingly, it could be a positive relationship between the intensity of solar radiation and the quantity of phenollics produced by plants (Niknam and Ebrahimzadeh, 2002). A rise in total phenolics was generally found in plants grown in sunny situations relative to shady ones, and such relationship could also be seen at the intra-individual level by comparing plant parts exposed to different amounts of light. It seems that rainfall scarcity and long light exposure may be involved in the activation of phenol biosynthesis (Naczk and Shahidi, 2006). Actually, plant species have inherent physiological differences, as a result of interactions with their environment (Taulavuori et al., 2010). These differences may be reflected by the presence of various chemical compounds that provide information regarding the ecotype conditions (Taulavuori et al., 2010). Moreover, BFA displayed the highest polyphenol content and the lowest antiradical activity; which may probably depend in one hand on phenolic nature rather than quantity and in other hand, on fatty acid composition. Previous results in cactus pear, Galati et al. (2003) analyzed *O. ficus- indica* (L.) Mill. juices (95% yellow and 5% red cultivars) and they identified the presence of major flavonoids, isoharmnetin triglycoside, rutin and kaempferol, as the main water-soluble constituents. Total flavonoid content corresponds to 652.5 µg/ml and ascorbic acid concentration is 26.9 mg/100 ml of juice. Many types of flavonoids have been reported in *Opuntia* sp. and an important role has been proposed for their presence as the main responsible of the antioxidant power of its extracts (Lee and Lim, 2000). Their chemical structure and concentration are very variable and depend on the variety, ripening stages and the kind of tissue of the plants (Wallace, 1986) 3-O-glycosilated flavonols, dihydroflavonols, flavonones and flavonols have been found in Cactaceae plants and fruits (Kuti, 2000).

Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits, such as, red grape (Negro et al., 2003), vegetables (Luo et al., 2002) and medicinal plants (Bourgou et al., 2008) and shows that plant flavonoid levels can be influenced by environmental factors such as light, temperature, and mineral nutrition (Jaakola et al., 2002). Accordingly, flavonoid contents were higher in plants growing in more stressful climatic

conditions (Tunisia) than those coming from more appropriate environment (Texas). In this way, Rodrigues et al. (2011) suggested that the high levels of red onion flavonols are probably related to the high radiation and low rainfall during growing season. Moreover, Germ et al. (2010) showed that the subjection of Hypericum perforatum to increasing doses of UV radiation significantly increased its foliar flavonoids (from 6.5 to 9 g 100 g^{-1} DW). Several authors have reported a positive and significant relationship between the antioxidant components including phenols, polyphenols and tannins, respectively with the reducing power and DPPH radical scavenging capacity (Connan et al., 2006; Huang et al., 2005). It is extremely important to point out that, there was a positive correlation between antioxidant potential and phenolic content estimated by the Folin-Ciocalteu method (Ksouri et al., 2007) in other species. Nevertheless, these antioxidant and antimicrobial activities depend on phenolic nature, structure and synergistic interactions (Djeridane et al., 2006). In fact, phenolics were able to reduce damages induced in the photosynthetic systems by absorbing UV-radiation and they were produced to protect plants from stresses such as, long exposure to dryness and/or solar radiation (Macheix et al., 2005; Wahid and Ghazanfar, 2006). In literature, phenolic compounds are assumed to directly contribute to antioxidative action since their level is strongly correlated with in vitro-measured antioxidant activities (Duh and Yen, 1999; Falleh et al., 2008).

Overall, the literature describes that antioxidant capacities are more variable in plants of different species (inter-specific) than within the same species (intra-specific). Accordingly, previous studies had shown that the purple-skinned cactus pear varieties of *Opuntia lindheimeri* (from Texas) had the highest total flavonoids (93.5 ± 12.4 mg/g of fruit), followed by the green-skinned of *O. ficus-indica* (69.5 ± 3.8 mg/g of fruit), the red-skinned of *Opuntia streptacantha* (54.8 ± 5.1 mg/g of fruit) and the yellows skinned of *Opuntia stricta var. srticta* (9.8 ± 3.0 mg/g of fruit), respectively (Kuti, 2004).

Conclusion

The data obtained will be important as an indication of the potentially nutraceutical and economic utility of cactus pear as a new source of fruit oils and functional foods. Moreover, these results provide useful information for the industrial application of *O. ficus-indica* fruits. Thirteen fatty acids were identified in fruit extracts oleic, linoleic and palmitic acids were the major fatty acids. Furthermore, this species displayed a high antiradical and antibacterial activity; and possessed a high phenolic content as compared to other medicinal plants and fruits (e.g. grape or apple) or beverages (tea) well known for their richness in polyphenols. This make *O. ficus-indica* a novel natural source of antioxidants with numerous health benefits. Besides, purified components from fruits may be used as natural antimicrobials in food systems. This need to be confirmed in future studies.

REFERENCES

Arcoleo A, Ruccia M, Cusmando S (1961). Flavonoid pigments from *Opuntia*. I. Isorhamnetin from flowers *Opuntia ficus-indica*. Annal Chimica (Rome). 51:81.

Balasundram N, Sundram K, Sammar S (2006). Phenolic compounds in plants and agri-industrial by- products. Antioxidant activity occurrence and potential uses. Food Chem. 1:191-203.

Barbera G, Carimi C, Inglese P (1992). Past and present role of the Indian-fig, prickly pear, (*Opuntia ficus-indica* (L.) Miller, Cactaceae), in agriculture of Sicily. Econ. Bot. 46:10-20.

Barbera G, Inglese P (1993). La coltura del ficodindia. Edagricole, Bologna. p. 188.

Barbera G, Inglese P, Pimienta-Barrios E (1995) Agroecology, cultivation and uses of cactus pear. FAO Plant Production and Protection. Rome. Paper 132:216p.

Bockisch M (1998). Vegetable fats and oils. In M. Bockisch (Ed.), Fats and oils handbook (pp. 174-344). Champaign:AOCS Press.

Bourgou S, Ksouri R, Bellila A, Skandrani, I, Falleh H, Marzouk B (2008). Phenolic composition and biological activities of Tunisian Nigella sativa L. shoots and roots. Compte Rendu de Biologies. 331:48-55.

Connan S, Deliste F, Deslandes E, Gall E (2006). Intra-thallus phlorotannin content and antioxidant activity in Phaeophyceae of temperate waters, Bot. Marine 49:39-46.

Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, Wyllie KSG (2000). The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil). J. Appl. Microbiol. 88:170-175.

Dewanto X, Wu KK, Adom R, Liu H (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity, J. Agric. Food Chem. 50:3010-3014.

Ennouri M, Bourret E, Mondolot L, Attia H (2005). Fatty acid composition and rheological behaviour of prickly pear seed oils. Food Chem. 93:431-437.

Falleh H, Ksouri R, Chaieb K, Karray-Bouraoui N, Trabelsi N, Boulaaba M, Abdelly C (2008). Phenolic composition of Cynara cardunculus L. organs, and their biological activities. Comptes Rendue de Biologie. 331:372-379

Griffiths R, Hare F (1907). The tuna as a food for man. USDA Bur. Plant Industry Bulletin. 116.

Gurrieri L, Miceli, C, Lanza MF, Tomaselli R, Bonomo P, Rizzarelli E (2000). Chemical characterization of Sicilian prickly pear (*Opuntia ficus-indica*) and perspectives for storage of its juice. J. Agric. Food Chem. 48:5424-5431.

Huang D, Ou B, Prior RL (2005). The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53:1841-1856.

Ibañez-Camacho R, Roman-Ramos R (1979). Hypoglycemic effect of *Opuntia* cactus, Arch. Invest. Med. (Mex). 10:223-230.

Inglese P, Barbera G, La Mantia T (1995). Research strategies and improvement of cactus pear (*Opuntia ficus-indica*) fruit quality and production. J. Arid Environ. 29:455-468.

Jaakola L, Määttä K, Pirttilä AM, Törrönen R, Kärenlampi S, Hohtola A (2002). Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanidin, and flavonol levels during Bilberry fruit development. Plant Physiol. 130:729-739.

Kamath V, Rajini PS (2007). The efficacy of cashew nut (Anacardium occidentale L.) skin extract as a free radical scavenger. Food Chem. 103:428-433.

Kamel BS, Kakuda Y (2000). Fatty acids in fruits and fruit products. In C. K. Chow (Ed.), Fatty acids in foods and their health implications (2nd ed.) New York: Marcel Dekker. 239-270.

Koleva II, Teris AB, Jozef PH, Linssen AG, Lyuba NE (2002). Screening of plant extracts for antioxidant activity:a comparative study on three testing methods Phytochem. Anal. 13:8-17.

Kumaran A, Karunakaran RJ (2007). *In vitro* antioxidant activities of methanol extracts of five Phyllanthus species from India.

Lebensmittel-Wissenschaft und Technologie. 40:344-352.
Ksouri R, Falleh H, Megdiche W, Trabelsi N, Hamdi B, Chaieb K, Bakhrouf A, Magné C Abdelly C (2009). Antioxidant and antimicrobial activities of the edible medicinal halophyte Tamarix gallica L. and related polyphenolic constituents. Food Chem. Toxicol. 47:2083-2091.
Kuti JO (1992). Growth and compositional changes during development of prickly pear fruit. J. Horticult. Sci. 67:861-868.
Kuti JO (2000). Antioxidant activity of Opuntia cactus pear. HortScience. 35, 433.
Kuti JO (2004). Antioxidant compounds from four *Opuntia* cactus pear fruit varieties. Food Chem. 85:527-533.
Lee EB, Hyun JE, Li DW, Moon YI (2001). The effect of *Opuntia ficus - indica* var. saboten fruit on gastric lesion and ulcer in rats. Nat. Prod. Sci. 7:90-93.
Lee EB, Hyun JE, Li DW, Moon YI (2002). Effects of *Opuntia ficus-indica* var. *Saboten* stem on gastric damages in rats, Arch. Pharm. Res. 25:67-70.
Lee JC, Kim J, Jang YS (2002). Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica* var. Saboten. J. Argic. Food Chem. 50:6490-6496.
Lee JC, Lim KT (2000). Effects of cactus and ginger extract as dietary antioxidants on reactive oxidant and plasma lipid level. Food Sci. Biotechnol. 9:83-88.
Lee K, Lim T (2000). Effects of cactus and ginger extract as dietary antioxidants on reactive oxidant and plasma lipid level. Food Sci. Biotechnol. 9:83-88.
Lee NH, Yoon JS, Lee BH, Choi BW, Park KH (2000). Screening of [the radical scavenging effects, tyrosinase inhibition, and anti-allergic activities using *Opuntia ficus-indica*, Kor. J. Pharmacogn. 31:412-415.
Lisiewska Z, Kmiecik W, Korus A (2006). Content of vitamin C, carotenoids, chlorophylls, and polyphenols in green parts of dill (*Anethum graveolens* L.) depending on plant height, J. Food Comp. Anal. 19:134-140.
Liyana-Pathirana CM, Shahidi F (2006). Antioxidant properties of commercial soft and hard winter wheats (Triticum aestivum L.) and their milling fractions. J. Sci. Food Agric. 86 (3):477-485.
Luo XD, Basile MJ, Kennelly EJ (2002). Polyphenolic antioxidants from the fruits of Chrysophyllum cainito L. (star apple). J. Agric. Food Chem. 50:1379-1382.
Maataoui BS, Hmyene A, Hilali S (2006). Activités anti-radicalaires d'extraits de jus de fruits du figuier de barbarie (*Opuntia ficus-indica*). Lebanese Science Journal, Vol. 7, No. 1.
Macheix JJ, Fleuriet A, Jay-Allemand C (2005). Les composés phénoliques des végétaux, un exemple de métabolites secondaires d'importance économique. Presses Polytechniques et Universitaires Romandes (Eds), Lausanne.
Maisuthisakul P, Suttajit M, Ponsawatmanit R (2007). Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants, Food Chem. 4:1409-1418
Mann JD (2004). Folts, Pathophysiology. 10- 105.
Mariod AA, Matthaus B (2008). Investigations on fatty acids, tocopherols, sterols, phenolic profiles and oxidative stability of Cucumis melo var. agrestis oil. J. Food Lipids. 15 (1):42-55.
Morales M (2007). Compuestos bioactivos en ecotipos coloreados de tuna y elaboración de una salsa para postre. Tesis para optar al grado de Magister en Ciencias Agropecuarias. Mención Producción Agroindustrial. Facultad de Ciencias Agronómicas, Universidad de Chile.
Naczk M, Shahidi F (2006). Phenolics in cereals, fruits and vegetables:occurrence, extraction and analysis. J. Pharm. Biomed. Anal. 41:1523-1542.
Negro C, Tommasi L, Miceli A (2003). Phenolic compounds and antioxidant activity from red grape marc extracts. Bioresource Technology. 87:41-44.
Niknam V, Ebrahimzadeh H (2002). Phenolic content in Astragalus species. Pak. J. Bot. 34(3):283-289.
Odoux L, Dominguez A (1996). Le figuier de Barbarie:une source industrielle de bétalaïnes *Fruits*. 51(1):61-78.
Oyaizu M (1986). Studies on products of the browning reaction prepared from glucose amine. Japan. J. Nutr. 44:307-315.
Parck EK, Kahng JH, Paek EA (1998). Studies on the pharmacological actions of cactus:identification of its anti-inflammatory effect. Arch Pharm Res. 21:30-34.
Parish P, Felker P (1997). Fruit quality and production of cactus pear (*Opuntia* spp) clones selected for increased frost hardiness. J. Arid Environ. 37:123-143.
Pimienta E (1990). El Nopal Tunero. Univ. Guadalajara Publisher, Guadalajara, Mexico. 246 pp.
Pimienta-Barrios E (1994). Prickly pear (*Opuntia* spp.):a valuable fruit crop for semi-arid lands of Mexico. J. Arid Environ. 28:1-11.
Rodrigues AS, Pérez-Gregorio MR, García-Falcón MS, Simal-Gándara J, Almeida DPF (2011). Effect of meteorological conditions on antioxidant flavonoids in Portuguese cultivars of white and red onions. Food Chem. 124:303-308.
Saenz ES, Araya E, Calvo C (1993). Colour changes in concentrated juices of prickly pear (*Opuntia ficus-indica*) during storage at different temperatures. Lebensm. Wiss. U. Technol. 26:417-421.
Sanchez-Moreno C (2002). Methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Sci. Technol. Int. 8:121-137.
Sawaya H, Khatchadourin W, Safi H, Al-Muhammed M (1983). Chemical characterization of prickly pear pulp, *Opuntia ficus-indica* and manufacturing of prickly pear jam. J. Food Technol. 18:183-193.
Sawaya WN, Khan P (1982). Chemical characterization of prickly pear seed oil, *Opuntia ficus-indica*. J. Food Sci. 47:2060-2061.
Simic MG (1988). Mechanisms of inhibition of free-radical processed in mutagenesis and carcinogenesis. Mutation Res. 202:377-386.
Soares JR, Dins TCP, Cunha AP, Ameida LM (1997). Antioxidant activity of some extracts of Thymus zygis. Free Radical Res. 26:469-478.
Stintzing F, Carle R (2004). Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. Trends in Food Science and Technology. 15:19-38.
Sun J, Richardo-da-Silvia M, Spranger I (1998). Critical factors of vanillin assay for catechins and proanthocyanidins. J. Agric. Food Chem. 46:4267-4274.
Taulavuori E, Taulavuori K, Niinimaa A, Laine K, (2010). Effect of ecotype and latitude on growth, frost hardiness, and oxidative stress of south to north transplanted scots pine seedlings. International Journal of Forestry Research. doi:10.1155/2010/162084.
Teles J, Stull W, Brown F, Whitting (1984). Amino and organic acids of prickly pear cactus (*Opuntia ficus-indica* L.). J. Sci. Food Agric. 35:421-425.
Tesoriere L, Fazzari M, Allegra M, Livrea MA (2005). Biothiols, taurine, and lipid-soluble antioxidants in the edible pulp of sicilian cactus pear (*Opuntia ficus-indica*) fruits and changes of bioactive juice components upon industrial processing. J. Agric. Food Chem. 53:7851-7855.
Trejo-Gonzalez A, Gabriel-Ortiz G, Puebla-Perez AM, Huizar-Contreras MD, Munguia-Mazarigos MR, Mejia-Arreguins CE (1996). A purified extract from prickly pear cactus (*Opuntia fulginosa*) controls experimentally induced diabetes in rats, J. Ethnopharmacol. 55:27-33.
Wahid A, Ghazanfar A (2006). Possible involvement of some secondary metabolites in salt tolerance of sugarcane. J. Plant Physiol. 163:723-730
Wallace RS (1986). Biochemical taxonomy and the Cactaceae. Cactus Succulent J. (USA). 58:35-38.
Wessels (1988). Spineless PricklyPears. Perskor, Johannesburg, South Africa. 61pp.
Wie MB (2000). Protective effects of *Opuntia ficus-indica* and *Saururus Chinensis* on free-radical induced neuronal injury in mouse cortical cell cultures, Yakhak Hoiji. 44:613-619.