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RESEARCH ARTICLE

Opuntiaficus Indica flower: A prominent source of phenolic compounds and unsaturated fatty acid

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Abstract

Comparison of the total phenol content and the antioxidant activities of three cultivars of *Opuntiaficus-indica* (L.) Mill. have been evaluated. The results showed that total phenolic compounds were abundant in flowers of all cultivars, the most abundant was found in Gialla cultivar (6.96 mg EGA/g DM). Quite the opposite, the antioxidant activity of the flower extracts was stronger in the Bianca cultivar than the other cultivars. Accordingly, Gialla flower extract exhibited the highest flavonoid content (4.29 mg CE/g DW) followed by Bianca (2.48 mg CE/g DW) and Rossa (1.64 mg CE/g DW). Likewise, Gialla flower extract exhibited the highest anthocyan content (0.30 mg / 100g FW) followed by Rossa (0.24 mg / 100g FW) and Bianca (0.16 mg / 100g FW). The same tendency was observed for the amount of tannins. The Rossa flower extracts displayed the highest DPPH scavenging (55.17%) followed by Gialla cultivar (54.73%) and Bianca cultivar (52.80%). Furthermore, flower extracts showed appreciable antibacterial properties against human pathogen strains. The mean inhibition zone was from 8.33 to 11 mm when the concentration was 50 mg/l. The strongest activity was recorded against *Enterococcus facium* and the lowest activity was observed against *Candida albicans*. Whereas, flower extracts were efficient to inhibit growth of pathogenic bacteria mainly against *Staphylococcus aureus* and *Echerchia coli*.

The data indicate that *Opuntiaficus-indica* flower is a rich source of natural antioxidants for foods. Subsequently compositions and concentrations of fatty acids, total lipids recovered were found to be 4.84 % (on dry weight). Among the total lipids, linolenic and palmitoleic acid were the dominating fatty acid and were estimated to be in relatively equal amounts 26.37 and 27.05 % respectively followed by linoleic and oleic acid. These findings suggest that *Opuntiaficusindica* flower may be considered as an interesting source of antioxidants for therapeutic or nutraceutical industries and for food manufactures.

INTRODUCTION

With 1600 species (Barthlott and Hunt, 1993) of the New World, the Cactaceae is one of the most distinctive and successful families of plants among angiosperms. Because flowers and fruits of *Opuntia* are strongly associated with pollination and seed dispersal by animals (Gibson and Nobel, 1986; Valiente-Banuet et al., 1996), the success of the group might be related to its distinctive reproduct**i**ve biology.

Most *Opuntia* species produce flowers and cladodes throughout their life-span, but flower and cladode production can alternate or fluctuate from one year to the next (Bowers, 1996a).
In subtropical regions, *Opuntia* floral-buds start when the mean monthly temperature exceeds 16 1C, in March or April in the northern hemisphere and in September or October in the southern hemisphere (Nerd and Mizrahi, 1995). The period of floral bud production is long, fluctuating from 3 to 5 weeks in *O. ficus-indica* (Wessels and Swart, 1990).*Opuntiaficus-indica* develops up to 20 floral buds per cladode (Nerd and Mizrahi, 1995).*Opuntia flowers show* generally yellow petals, but there are also orange, pink, purple, red, white or mottled flowers (Anderson, 2001; Bravo, 1978).

Proper utilization of this by-product could reduce waste disposal problems and serve as a potential new source of fats and proteins for use in food and feed (Kamel&Kakuda, 2000).
Previous remote studies on the plant flowers reported the isolation of b-sitosterol, fatty acids, and some of their esters from an apolar extract (Arcoleo et al., 1966), and an unidentified isorhamnetin glycoside (Arcoleo et al., 1962). Recent work on Egyptian cultivated cactus, reported the isolation and identification of penduletin, kaempferol, luteolin, quercitrin, and rutin (El- Moghazy et al., 1984). From bibliographic information a correlation between diuretic activity and high content of potassium was evidenced (Battaglini, 1939), whereas Galati et al. (2002) demonstrated in rats this action for waste matter such as cladodes, flowers, and un- marketable fruit infusions. In the traditional Italian medicine (Sicily), the infusion of flowers is known as a diuretic agent. Other medicinal uses have been reported by Monjauze and Le Houerou (1965) for North Africa, where the flowers are used to combat amoebic dysentery.

The potential health benefits of phenolic compounds have been widely reported (Santos-Buelga et al., 2003). They are strong antioxidants and free radical scavengers which inhibit oxidation of lipids (Lee et al., 2006). In addition; they have anti-inflammatory, antibacterial and antifungal properties(Pettinari et al., 2006).The increasing interest of nutritional and pharmacological power of different parts of *O. ficus-indica*, also at industrial level, motivated our investigation on the chemical content of the flowers, which are less known despite the copious literature reported on the fruits. Therefore, to provide major information about the chemical content of *O. ficus-indica* flowers, we performed a qualitative and quantitative analysis of fatty acid of plant material from the Mediterranean area (Tunisia). Also the secondary metabolites (phenolic compounds) of the methanol extract and their biologic activities never reported before were also determined.

In that framework, 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 *Opuntiaficusindica* flower.

Material and methods

Plant material

Flowers of *Opuntiaficus-indica* were collected(Figure 1 (A))at the end of their development in Kasserine (Centre-West of Tunisia), in Mach 2010,were rinsed with distilled water and then oven dried All harvested samples were grinded to fine powder(Figure 1 (B))and stored at- 20°Cuntil analysis began. Open flowers include the inferior ovary and the perianthium.

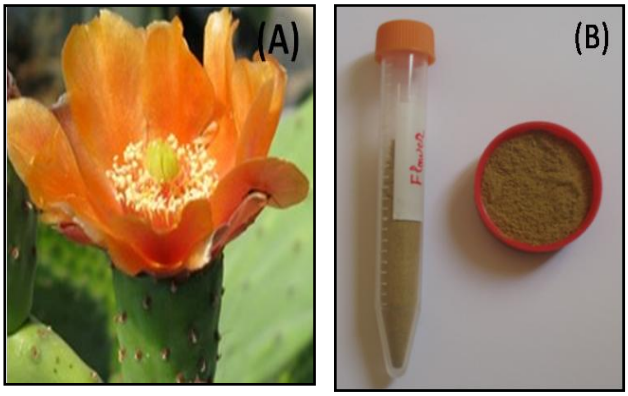

Figure 1: (A) Flowers of *Opuntiaficusindica*. (B) Powders obtained from flowers after freeze-drying.

Preparation of plant extract

Sample extracts were obtained by magnetic stirring of 2.5 g of dry organ 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 Whatman No4 filter paper, and evaporated under vacuum to dryness. They were stored at 4°C until analysis began.

Extraction of total lipid

Soxhlet extraction apparatus and procedure

For conventional Soxhlet extraction of oil from cactus the ISO 659-1988 (E) procedure was used. Soxhlet extractions were performed using 40 g of cactus sample after grinding in an electrical mill. The amount was transferred in a (33 mm×100 mm) cellulose thimble and placed after in the extraction chamber of a 200ml capacity Soxhlet apparatus.

The cellulose thimble was plugged with cotton in order to avoid transfer of sample particles in the distillation flask. The Soxhlet apparatus, fitted with a condenser, was placed on a 500ml distillation flask containing 300ml of solvent and 3 boiling glass regulator. Samples were thus extracted under reflux with *n*-hexane during 4 h (18–22 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 during 2 until a total extraction of 8 h (4h+2h+2h).

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 for 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:

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

Analysis of fatty acid composition

Fatty acid methyl esters (FAMEs) of the studied oil samples were prepared based on a method described by Stéfanoudaki et *al*. (1999).Briefly, 0.1 g of oil was weighed in a 20 ml test tube (with screw cap). The sample was dissolved in 3ml of hexane, and 0.5ml of potassium hydroxide (2N) in methanol was added. The test tube was capped and vortexes for 30 seconds. The mixture was centrifuged at 402 × *g* for 10 min, and the extracts was transferred to a 2 ml autosampler vial, and analyzed using gas chromatography.

Gas chromatography (GC) condition

Fatty acid composition of the studied 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. 0.25μmpolythylene 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 ˚C 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 assays

Evaluation of total antioxidant capacity

The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH (Prieto et al., 1999). An aliquot (0.1 ml) of sample extract was combined in an eppendorf tube with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate).The tubes were incubated in a thermal block at 95°C for 90 min. After, the mixturehad cooled to room temperature; the absorbance of each solution was measured at695 nm (Anthelie Advanced 2, SECOMAN) against a blank. The antioxidant capacitywas expressed as mg gallic acid equivalent per gram of dry weight (mg GAE/g DW). The calibration curve range was 0–500 µg/ml. All samples were analyzed in triplicate.

DPPH radical-scavenging activity

The DPPH quenching ability of plant extracts was measured according to Hanatoetet al., 1988). One ml of the extract at different concentrations was added to 0.5 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 and corresponded to the ability of extracts to reduce the stable radical DPPH to the yellow-colored diph-enylpicrylhydrazine. The antiradical activity was expressed as inhibition percentage. The ability to scavenge the DPPH radical was calculated using the following equation**:**
DPPH scavenging effect $AA\ (\%) = [(A_0 - A_1)/A_0] \times 100$ Eq. (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.

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 deionizer 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. Reducing power activity was expressed in µ/ml as inhibition percentage.

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 and 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. Sample extract and reference compounds (BHT and BHA) were prepared in ethanol. An aliquot (150 µl) of the β-carotene: linoleic acid emulsion was distributed in each of the wells of 96-well microtitre plates and ethanolic solutions of the test samples (10 µl) were added. Three replicates were prepared for each of the samples. The microtitre plates were incubated at 50°C for 120 min, and the absorbance was measured using a model EAR 400 microtitre reader (LabsystemsMultiskan 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:

$AA\ (\%) = [(A_1 - A_0)/(A_0 - A_1)] \times 100$ Eq. (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 as inhibition percentage (%).

Screening for antimicrobial activity

The antibacterial activity of flowers extracts was assessed by the agar disk diffusion assay (Bagamboula et al., 2003) against four human pathogenicbacteria: Gram-positive cocci including *Staphylococcus aureus* (ATCC 25923) and Gram-negative bacteria including *Escherichia coli* (ATCC35218),*Enterococcus facium*, and*Salmonelletyphi*.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 suspensionmedium (BioMérieux) and adjusted to the 0.5 McFarland turbidity standardwith a Densimat (BioMérieux). A sterile filter disc with 6 mm in diameter (Whatman 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 for1 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 2005guidelines. Discs with 10 µl of pure methanol were used as negative controls. For the antifungal activity of the same organ extracts, 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 18–24 h. Several colonies were transferred into Api suspension medium and adjusted to two McFarland turbidity standard. The inoculate of the respective yeasts were streaked onto Sabouraud chloramphenicolagar plates at 30°C using a sterile swab and then dried. A sterile filter disc, diameter6 mm (Whatman paper no. 3) was placed in the plate. Ten microlitres of extract concentration were dropped on each paper disc. The treated Petri dishes were placed at 4 °C for 1–2 h and then incubated at 37°C for 18–24 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 containing20 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

Quantification of anthocyan

Anthocyans are hydrosolubles flavonoids; their extractions were determined after incubation for 72 hours at 4°C in darkness. 200 mg of fresh matter was dissolved in 2 ml of HCl-H_2O-Methanol in proportion (1v, 3v, 16v). The absorbance was measured at 530 and 653 nm (Gould et al., 2000); and the solvent was used as a positive control. Anthocyans content was calculated using the following equation**:**

Anthocyans ($\mu g.ml^{-1}$) = $A_{530} - 0.24\ A_{653}$ Eq. (3)

Determination of total polyphenol content

Colorimetric quantification of total polyphenols was determined, as described by Dewanto et al. (2002). An aliquot of 125 µl of diluted extract were added to500 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. A standard curve of gallic acid was used. Total phenolic content of organs 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 analyzed in triplicate.

Estimation of total flavonoid content

Total flavonoids were measured by a colorimetric assay according to 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 a blank.

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 analyzed in triplicate

Quantification of total condensed tannins
The analysis of condensed tannins (Proanthocyanidins) was carried out according to the method of Sun et al. (1998). 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. 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 ofcatechin was established between 0 and 400 µg/ml. All samples were analyzed in triplicate.

Statistical analysis
For all plant parameters, three replicates were used. To determine the relative importance of cultivars and organs, and their interaction on phenolic content, antioxidant activities and antibacterial activities, a two-way 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 and discussion

Total antioxidant capacity

Global antioxidant activity of *Opuntiaficusindica* flowers was expressed as the number of gallic acide quivalents. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo(V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm. The study reveals that the antioxidant activity of Gialla flower extract was 1.81-fold higher than that of the Bianca extract and 3.35-fold higher than that of the Rossa extract (Table 1). This strong antioxidant activity of Gialla flowers (13.36 mg GAE/g DW) might be attributed to the presence of phytochemicals such as phenolic compounds. 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).

Table 1: Effect of the cultivars on total antioxidant capacity and on phenolic compounds in *Opuntiaficusindica* flowers using an analysis of variance (ANOVA). Values are means of three replicates.

Anthocyan content (mg/100g FM)	Condensed tannin contents (mg of CE/gDW)	Flavonoïd contents (mg of CE/gDW)	Polyphenol contents (mg of GAE/gDW)	Total antioxidant capacity (mg of GAE/g DW)	Flower cultivars
0.24	0.05	1.64	4.85	3.98	Rossa
0.30	0.35	4.28	6.96	13.36	Gialla
0.16	0.19	2.48	4.93	7.37	Bianca

Values followed by one or more of the same letters were not significantly different at $P<0.05$ according to the Newman-Keuls post-hoc test.

Analysis and quantification of phenolic compounds

Total phenolics contents in *Opuntiaficusindica*

Natural antioxidants such as polyphenols are often added to foods to stabilize them and prevent off-flavor development and have considerable interest for their potential role as functional foods or nutraceuticals (Espin et al., 2007). In fact, phenolics may contribute to the bitterness, astringency, color, flavor, odor and oxidative stability of food (Naczk&Shahidi, 2006.Indeed, phenolic compounds have been shown to protect the plant cell wall during times of UV, salt, or pathogenic stress (Strycharz&Shetty, 2002; Alasalvar*et al.* 2005). As previously reported for Gialla cactus pear (Piga et al., 2003), the polyphenols declined significantly after six days of storage at 41°Cand for tomato (Toor*et al.* 2006), the contents in antioxidant compounds are influenced by the environmental parameters (solar radiation, temperature, rainfall, and edaphic factors) during plant development, and thus vary at different times of the year.

Opuntiaficusindica flowers are good, inexpensive water source. Content water is about 79.86% for fresh weight.

The amounts of total phenolics in the *Opuntiaficusindica* flower are summarized in Table 1.The contents of total phenolic compounds in different cultivar flower were significantly decreased in the order of Gialla> Bianca>Rossa. These findings indicate that secondary metabolites distribution may fluctuate between different plant cultivars. Alternatively, it wasrevealed that phenolic contents varied greatly among the cactus cultivars. Total phenolics contents ranged from as low as 4.85mg EAG/g DW in Rossa flower cultivar to as high as 6.96mg EAG/g DW in Gialla flower cultivar.

This is in agreement with other results in cactus pear (Saenz et al., 1993; Odoux & Dominguez, 1996). They were significantly decreased in the order of Rossa> Bianca>Gialla.

In a nutshell, the high content of total phenols in all methanolic extracts might explain the strong antioxidant properties of these cultivars. We are in general agreement with Inglese et al. (1995) on the ideal cactus cultivar but we would add the following attributes; mature yield, post harvest shelf life (Felker et al., 2002a) and in a variety of colors (yellow, orange, pink and purple).

Furthermore, a linear relationship existed between concentration and total phenolic content, flavonoid and condensed tannin. The corresponding correlation coefficients were ($y = 0.004x + 0.066$) and *0.9977* ($y = 0.0033x + 0.0184$) for phenolic content, flavonoid and condensed tannin, respectively.

Total flavonoid content of *Opuntiaficusindica* extracts

The most important classes of phytochemicals in plant foods are phenolics and there are more than 8000 phenolic phytochemicals. The three main classes of dietary phenolics are flavonoids, and phenolic acids (Vinson et al., 2001). The flavonoids constitute about one-half of the 8000 or so recognized phenols and are molecules responsible for the color of fruit and flowers (Cook &Samman, 1996).

As phenolics contents, the same tendency was observed for flavonoid content. Flavonoid contents in the three cactus pear fruit varieties are shown in Table. Flavonoid content varied significantly greatly among the cactus cultivars examined in this study. Total flavonoid contents ranged from as low as 1.64 mg CE/g dry weight in Rossa flower cultivar and as 2.48 mg CE/g dry weight in Bianca flower cultivar to as high as 4.28 mg CE/g dry weight in Gialla. These results advise that flavonoid content of *Opuntiaficusindica* was influenced by the cultivar.

Compared with other *Opuntia* species studied by Kuti, (2004), (the red-skinned cactus pear: 54.8 µg/g fresh wt from *Opuntiastreptacantha* and the yellow skinned: 9.8 µg/g fresh wt from *Opuntiastricta*), *Opuntiaficusindica* skin had the highest total flavonoid (yellow skinned: 1.21 mg CE/g dry weight)(Table 1).

It appears that *Opuntia* cactus pear fruits contain flavonoids common to other fruits and vegetables (Peterson & Dwyer, 1998); however, the flavonoid types and contents, as in other fruits and vegetables, vary with the cultivars (Bilyk&Sapers, 1986; Howard et al., 2002).

Condensed tannins in methanolic extract

A low condensed tannins content varied significantly and merely among the *Opuntiaficusindica* cultivars (Table 1).They were present in all studied plant samples, even though in lower abundance than flavonoids, (Table).The amount of tannin was higher in Gialla flower (0.35 mg CE g−1 DW) than in Bianca flower (0.19 mg CE g−1 DW) and in Rossa flower (0.05 mg CE g−1 DW).

Totalanthocyanscontent

Recent research results have shown that anthocyanins from edible fruits were effective antioxidants in vitro (Einbond et al., 2004). Antioxidative properties of anthocyanins arise from their high reactivity as hydrogen or

electron donors, and from the ability of the polyphenol derived radicals to stabilize and delocalize the unpaired electron, and from their ability to chelate transition metal ions (termination of the Fenton reaction) (Rice-Evans et al., 1997). All *Opuntiaficusindica* cultivars were found to contain anthocyan (Table 1).
Based on the results, the total concentration of anthocyans was higher in Gialla flower (0.30 mg/100 g FW) than in Rossa flower (0.24 mg/100 g FW) and in Bianca flower (0.16 mg/100 g FW). Besides, Anthocyan content varied significantly greatly among the cactus varieties**.**

Antioxidant activity in *Opuntiaficusindica* flowers
DPPH radical-scavenging activity

Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid peroxidation (Rice-Evans et al., 1997; Bloknina et al., 2003). The DPPH radical-scavenging activity has been extensively used for screening antioxidants from fruit and vegetable juices or extracts (Robards et al., 1999; Sanchez-Moreno, 2002).
In this study, the reduction capability of DPPH radical was determined by the decrease in absorbance induced by plant antioxidants. BHT (79.75%) was the reagent used as standard. The experimental data of these cultivars reveal that organ extracts have a stronger effect of scavenging free radical.
The methanolic extract significantly inhibited the activity of DPPH radicals.Rossa, Gialla and Bianca cultivarshad high scavenging activity: 55.17, 54.73 and 52.80% respectively (Table 2). Nevertheless, this scavenging activity decrease when extract concentration increase (100 to 1000 µg/ml). As a whole, these findings showed that ripening time had significantly and differently affect The DPPH radical-scavenging activity of *Opuntiaficusindica* flower. The scavenging effect of methanol extracts (1000 µg/ml) and standard on the DPPH radical increased in the following order: BHT>Gialla flower >Rossa flower > Bianca flower.

Table 2: Impact of the cultivars on antioxidant activity in *Opuntiaficusindica* flowers using an analysis of variance (Anova). Values are means of 3 three replicates.

Concentrations (µg.ml^{-1})						**Flowers cultivars**
BCBT test (%)**		**Reducing power (%)**		**DPPH* test (%)**		
1000	**100**	**1000**	**100**	**1000**	**100**	
39.52	2.06	0.20	0.08	35.96	55.17	**Rossa**
47.48	0.03	0.22	0.31	40.87	54.73	**Gialla**
68.73	7.58	0.16	0.12	22.28	52.80	**Bianca**

Values followed by one or more of the same letters were not significantly different at $P<0.05$ according to the Newman-Keuls post-hoc test.

Iron reducing power

It has been reported that reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Yen & Duh, 1993; Chang et al., 2002). As shown in Table 2 extracts from *Opuntiaficusindica* samples exhibited a lower reducing power than ascorbic acid (1.33%), suggestive of that they had weakness electron-donating capacity.
The ascorbic acid (positive control) concentration required to reduce the ferric iron was higher than flowers extracts, indicating a worse activity.
Actually, reducing power is a very important aspect for the estimation of the antioxidant activity (Ksouri et al., 2008). Reductones such as ascorbic acid can react directly with peroxides and with certain precursors, in that way preventing the formation of peroxide. Hence, the antioxidant activity of both plant extracts might be due to the reduction of superoxide anion, inactivation of free radicals or complexion with metal ions or combination of the three.

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 hamper the extent of β-carotene destruction by ‘‘neutralizing” the linoleate free radical and any other free radicals formed within the system (Kamath & Rajini, 2007). Table 2 shows the inhibition of β-carotene bleaching by the fruit and peel extracts of *Opuntiaficusindica* and by the two positive controls BHA (61.69%) and BHT (79.75%). In term of β-carotene bleaching effect, those samples exhibited the following order: BHT > Bianca flower >BHA>Gialla flower >Rossa flower.

Antimicrobial activity

Table 3shows the antibacterial activities of flowers measured by the agar diffusion method against selected pathogenic bacteria. The mean inhibition zone for all bacteria treated with *Opuntiaficusindica* flower extracts varied from 0 to 11 mm at 50 mg/l. Flower extracts were active against *E.faciu*mand *S.typhi*. Though, flower extracts were ineffective against *E.coli and S. aureus* (Table 3).

Table 3: Inhibitory effect of flower extracts on five human pathogen bacteria, compared to that of positive standard (gentamycin)

Diameter of growth inhibition (mm±SD)[a]		Bacterial strains
Flower extract	Gentamycin	
NA	22.23±0.66	*Echerchia coli* ATCC 35218
NA	18.3±0.6	*Staphylococcus aureus* ATCC 25923
9±0.5	24.7±0.60	*Salmonella thyphi*
11±0.5	22.95±0.66	Enterococcus facium
8.33±0.5	19.85±0.66	Candida albicans

[a] Data are reported as means ±SD of three measurements. NA: not active.

The strongest activity of *Opuntiaficusindica* flower was recorded against *E.facium* and the lowest activity was observed against *E. coli* and *S. aureus*. Concerning antifungal tests, flower extracts concentration (50 mg/ml) failed to show a strong activity against *C.albicans*. These results suggest that methanolic extracts of flower were more efficient to inhibit fungal growth than bacterial one, probably in relation to their active molecules.
Several studies attributed the inhibitory effect of plant extracts against bacterial pathogens to their phenolic composition (Baydar et al., 2004; Rodriguez Vaquero et al., 2007). The inhibitory effect of these phenolics could be explained by adsorption to cell membranes, interaction with enzymes, substrate and metal ion deprivation (Scalbert, 1991). These results suggest that the antibacterial capacity needed, as compared to antioxidant activity which has a good efficiency with crude extracts of *Opuntiaficusindica* flower, more concentration and even purification of phenoliccompounds. Purified components may be used as natural antimicrobialsin food systems, as well as to prevent the growth of foodborne bacteria resulting in extension of the shelf life of processedfoods.

Fatty acid compositions

Table 4 shows the retention time of authentic standards, typical CPG chromatogram of *Opuntiaficusindica* fruit extracts are presented in Figure 2, and the fatty acid compositions (%) of the studied oil samples are summarized in Table 5.

Table 4: Fatty acid standard retention time determine by CPG. RT: retention time.

Flower (%)	**Fattyacid**	
0	C12:0	
0.09	C14:0	
1.65	C16:0	
27.05	C16:1	
3.77	C17:0	
3.00	C18:0	11.90
10.56	C18:1	12.31
16.98	C18:2	13.24
26.37	C18:3	14.63
2.01	C18:4	15.26
2.93	C20:0	16.09
1.39	C20:1	16.60
3.19	C22:0	21.30
1.00	C22:1	21.91
4.83	TL	
14.63	SFA	
85.37	UFA	

Table 5: Fatty acid composition of *Opuntiaficusindica* flower

RT	Standards	
2.51	C8:0	1
3.79	C10:0	2
5.32	C12:0	3
6.09	C13:0	4
7.22	C14:1	5
7.78	C15:0	6
8.88	C16:0	7
9.25	C16:1	8
10.25	C17:0	9
11.90	C18:0	10
12.31	C18:1	11
13.24	C18:2	12
14.63	C18:3	13
15.26	C18:4	14
16.09	C20:0	15
16.60	C20:1	16
21.30	C22:0	17
21.91	C22:1	18

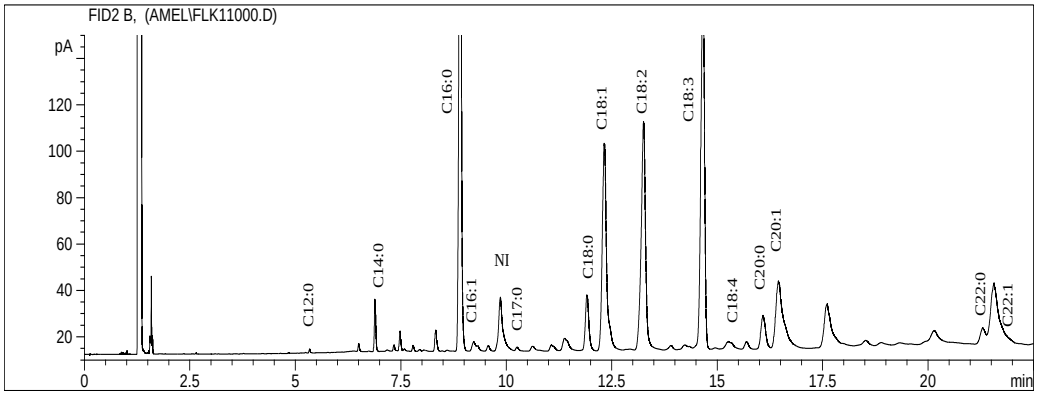

Figure 2: CPG chromatogram of the standards mixture of fatty acid methyl esters flowers
NI: non identified

Among the different categories of fatty acids, palmitoleic acid (16:1) (27.05%), oleic (18:1) (10.56%), linoleic (18:2) (16.98%) and linolenic acid (18:3) (26.37%) are the most abundant fatty acids (Figure 2).
The unsaturated fatty acids (UFA), is about 85.37%. In Malaysia, palm oil is commonly used, due to its superior frying quality and oxidative stability owing to high content of monounsaturated and saturated fatty acids (Ong&Goh, 2000). These findings show that flower oil may possess comparable nutritional content, as the palm oil, which has been extensively accepted and used as cooking oil in various food applications; in Malaysia, and many other countries. This information also suggests that flower oil could be an alternative cooking oil in the near future.
As recommended by the National Cholesterol Education Program/American Heart Association, C16:0 and C18:0 are best SFA from natural source (Hayes, 2002). Based on our results, high percentage of C16:0 (1.65%) and C18:0 (3%) in flower oil can be exploited as new source of vegetable oil. Additionally, the high palmitic (36%) and linoleic acids (12%) content of flower oil will be advantageous in hyperchlosterolicmic conditions as shown by Sundaram (2004) and Ng et al. (1994). They reported no hypercholesterolemic effect was observed when palmitic acid was consumed with linoleic acid above 4.5%. On the other hand, dabai kernel oil can be categorized as saturated fat-rich oil based on its fatty acid composition characterized by high SFA (60%) content. Besides, the MUFA content of the dabai kernel oil was about 36%, which is higher than soybean oil (22%) and corn oil (26.5%) (Baylina et al., 2007). Therefore, dabai kernel oil has high tendency to be solid at room temperature. Additionally, the fatty acid composition of the dabai kernel oil was similar to cocoa butter, which has 60% SFA, 36–37% MUFA and 3% PUFA (deMan&deMan, 1994).Kim et al. (2010) has reported values of 22 and 78% of saturated and unsaturated fatty acid composition from olive oil. These values are nearly similar to the results obtained in the present study. Baylina et al. (2007) have reported results of 48 and 37% of saturated and unsaturated fatty acids from palm oil, which is also similar to our findings.

Conclusion

The analysis of variance (ANOVA) for all data showed a significant influence on antioxidant capacities and phenolic contents of *Opuntiaficusindica*flowerfor each cultivar.
The highest phenolic contents (total polyphenol, flavonoid, anthocyan and condensed tannin) were obtained in Gialla flower extract.
A similar distribution was found for the capacities of flower extracts to quench DPPH, BCBT and Iron Reducing, being higher in Gialla cultivar than in the other.
Our findings suggest that anthocyan and flavonoid may be important antioxidant components in the flower.
On the basis of the results obtained, *Opuntiaficusindica* may play potential role as a source of health promoting phenolics and unsaturated fatty acid associated with antioxidant activity.
There is a high likelihood that *Opuntiaficusindica* flower may provide the types of nutritional and health benefit associated with consumption of fruits and vegetables in general. From this perspective it becomes evident that beneficial aspects of anthocyanins and polyphenols acid supporting human defense mechanisms are of increasing interest.
For that reason, in continuation of our previous work we investigate further studies on the absorption and effects of cactus flower phytochemicals on antioxidant status in animal models so as to evaluate their potential health benefit.

On the other hand, findings from the present study revealed good nutritional values of fats extracted from flower of *Opuntiaficusindica*. Information on the studied parameters is very useful in determining the opportunity of the oils to be used and commercialized as another source of vegetable oils and solid fats in future. The identification of new cooking oil and solid fat of *Opuntiaficusindica* indirectly adds variety to the types of vegetable oil and solid fats on the commercial market. Further work on the antioxidant activities of oils from *Opuntiaficusindica* fruit are worth investigation.

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