Solvent extract from *Opuntia stricta* fruit peels: Chemical composition and Biological activities

Mohamed Koubaa[1*], Ameni Ktata[1], Fatma Bouaziz[1], Dorra Driss[1], Raoudha Ellouz Ghorbel[1] and Semia Ellouz Chaabouni[1,2]

[1]*Enzyme Bioconversion Unit (UR13ES74), National School of Biological Engineering Department, Sfax University, Tunisia, AFRICA.*

[2]*Common Service Unit of Bioreactor coupled with an ultrafilter, National School of Biological Engineering Department, Sfax University, Tunisia, AFRICA.*

ABSTRACT

Background and aim: *Opuntia stricta* is belonging to the *Cactaceae* family. Its fruit is composed of around 69% peels, 21% pulp and 10% seeds. Extracting bioactive compounds from the peels; the major part usually discarded, helps to reduce the cost and environmental concerns associated with their disposal. Many studies have been interested in extracting dyes and phenolics from *O. stricta* peels, showing their antioxidant properties, but no work was devoted to extract and characterize the non-polar compounds meaning hydrodistillation. **Method:** *O. stricta* fruits were collected at ripening, the peels, pulps and seeds were manually separated. Peels were then blended, hydrodistilled, and the non-polar compounds were extracted and identified using gas chromatography–mass spectrometry. Afterwards, the antioxidant and antibacterial activities of the extracted molecules were investigated. **Results:** The extracted non-polar compounds from *O. stricta* fruit peels were mainly terpene alcohols. The major components were *trans*-linalool oxide, *cis*-linalyl oxide and linalool with 38.3%, 29.6% and 23.4%, respectively. The antioxidant activities showed high inhibition of the DPPH free radicals with 84% at 50 mg/ml, higher reducing power than that of ascorbic acid, and high total antioxidant activity with 309 ± 37 µg ascorbic acid equivalent at 25 mg/ml. The antibacterial activities showed high growth inhibition against *Staphylococcus aureus* and partial inhibition against *Enterococcus faecalis*. **Conclusion:** Non-polar compounds extracted meaning hydrodistillation from *O. stricta* fruit peels exhibit high antioxidant activities and inhibit the growth of *S. aureus*. They represent thus a promising way for the valorization of this by-product.

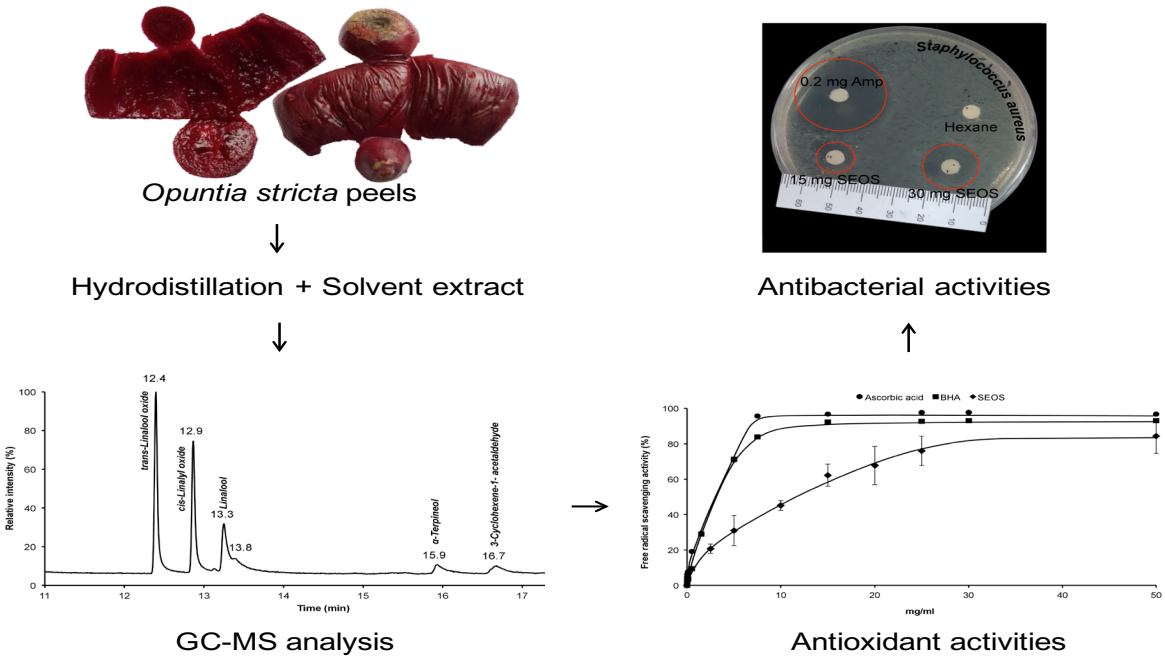
Key words: Antibacterial activities, Antioxidant activities, Non-polar compounds, *Opuntia stricta* fruit peels, Solvent extraction.

INTRODUCTION

Opuntia genus belongs to the *Cactaceae* family and comprises from 200 to 300 species.[1] *Opuntia* fruits are widely incorporated in confectionery specialities and cosmetics. However, for fruits, only the pulps and the seeds are usually used, and the thicky peels are generally discarded. Recently, extracting bioactive compounds from agro-industrial by-products has drawing attention of many research groups, revealing thus a way, among others, to reduce the cost and the environmental concerns associated with their disposal.[2–4] Numerous *Opuntia* species have been characterized for their fruit's peel bioactive compounds content, making them on the verge of commercialisation.[5–7] Other works have been reported in leterature describing the extraction of volatile compounds from *Opuntia* species, and studying their antioxidant activities.[8–11] Moreover, solvent extracts from some *Opuntia* species showed high antioxidant and

***Corresponding address:**
Mr. Mohamed Koubaa
Enzyme Bioconversion Unit (UR13ES74),
National School of Engineering P.O. Box 1173-3038, Sfax University, Tunisia, Africa.
E-mail: koubaa.mohamed@gmail.com

DOI: 10.5530/fra.2015.2.2



Graphical Abstract

antimicrobial activities (e.g. extracts from dried stem of *Opuntia dillenii*,[12] extracts from *Opuntia ficus indica* peels,[13] extracts from *Opuntia humifusa* stem,[14] etc). *O. stricta* Haw., a spineless prickly pear, is widely growing in the Mediterranean countries. Recently, Kunyanga co-workers 2015 evaluated the nutritional quality, phytochemical composition and health protective effects of *O. stricta* Haw. fuits, showing their benefits.[15] In Tunisia, this plant is ranked as the second widespread cactus growing after *O. ficus indica.* Its fruit peels, representing around the 2/3 of the fruit's weight, has taking attention for its valorization; e.g. the extraction and characterization of polyphenols, flavonoids, betacyanins,[16] and dyes.[17] In this line, this work has been conducted to analyze the phytochemical properties of solvent extracts from *O. stricta* Haw. (SEOS) fruit peels. For this purpose, non-polar compounds were extracted meaning hydrodistillation, and their composition has been assessed using gas chromatography–mass spectrometry. Afterwards, they were tested for their antioxidant activities through the DPPH free radical scavenging activity, the total antioxidant activity and the reducing power capacity. Moreover, their antibacterial activities were tested against eleven Gram + and Gram-pathogen strains.

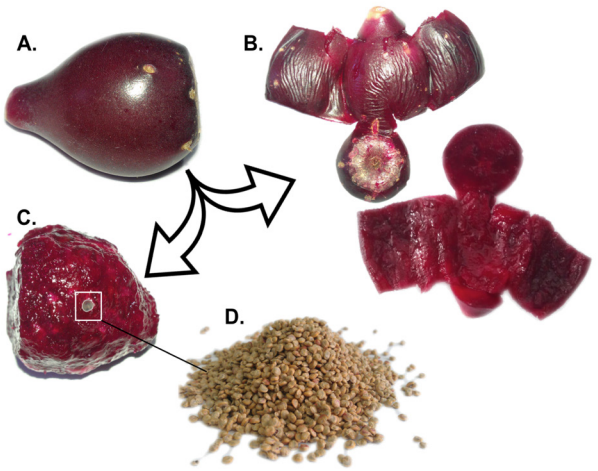


Figure 1: Composition of *Opuntia stricta* Haw. fruit. A. Fruit, B. Fruit peel, C. Fruit pulp, D. Seeds

MATERIALS AND METHODS

Chemicals

Ammonium molybdate was purchased from NenTech Ltd (United Kingdom). Potassium ferricyanide was obtained from Loba Chemie (India). DPPH (2,2-diphenyl-1-picrylhydrazyl), sodium phosphate, ferric chloride, and trichloroacetic acid were obtained from Sigma-Aldrich (France). Sulfuric acid and ethanol were obtained from Sharlab (Spain).

Plant material

O. stricta Haw. (*O. stricta* (Haworth) Haworth, Syn. Pl. Succ. 191. 1812.) fruits were collected on February 2014 in the suburb of Sfax city (Tunisia). All peels were manually separated from the pulps and the seeds (Figure 1), blended using a kitchen mixer until obtaining a viscous peel juice, then frozen at-20°C until analysis.

Non-polar compounds extraction

The method was adapted from Koubaa and co-workers (2015),[18] with slight modifications. Hydrodistillation was performed during 3 h using 200 g of *O. stricta* peel juice, submerged in 500 ml distilled water and boiled in a round bottom flask using heating mantle. The condensed distillate (~250 ml), consisting of a mixture of water, polar and non-polar compounds, was separated by adding 150 ml n-hexane in a separatory funnel. The solvent was then evaporated using a rotary evaporator system, under vacuum, at 30°C and the non-polar compounds extracted from *O. stricta* fruit peels (SEOS) were stored in glass vials at-20°C until analysis.

Chemical composition of SEOS

SEOS composition was analyzed by gas chromatography-mass spectrometry (GC-MS) using an Agilent Technologies (6890) instrument equipped with a capillary HP-5MS column (5% phenyl methyl siloxane, 30 m length, 250 µm diameter and 0.25 µm film thickness). The extracted SEOS was injected (1 µl) into the GC inlet and carried throughout the column using helium (1 ml/min) as carrier gas. The GC conditions were as follows: the initial oven temperature was set at 35°C, increased to 250°C at 5°C/min, and held for 6 min, allowing the separation of the molecules. The injection temperature was set at 250°C and the injection mode was set to "Splitless". The MS parameters were as follows: the interface temperature was set at 230°C with electron impact ionization and "full scan" modes between m/z 20 and 550. Peak's identification was based on Wiley library.

Antioxidant activities

Free radical scavenging activity

This spectrophotometric assay uses the stable free radical DPPH as reagent.[19] The antioxidant molecules are able to reduce the stable free DPPH radicals to a yellow colored 1,1-diphenyl-2-picrylhydrazyl. Free radical scavenging activity of SEOS was assessed as described previously,[20] with slight modifications. Briefly, 875 µl of SEOS, prepared in absolute ethanol, were mixed with 125 µl DPPH (0.02% in absolute ethanol) to get final concentrations ranging from 2.5 mg/ml to 50 mg/ml. The hydrogen or electron donation abilities of SEOS were measured, after 1 h incubation in the dark, by means of the decrease of the absorbance at 517 nm (A_{sample}) resulting in a color change from purple to yellow. Blank solutions were prepared under the same conditions without DPPH, the absorbance was measured as A_{blank}. The control solution was prepared by mixing 875 µl absolute ethanol with 125 µl DPPH and the absorbance was measured as $A_{control}$. The DPPH radical scavenging activity (percentage of inhibition) was then calculated as previously described.[18]

Total antioxidant activity

The total antioxidant activity of SEOS was performed as previously described,[18,21] with slight modifications. Different amounts of SEOS ranging from 2.5 mg to 25 mg were prepared in 100 µl absolute ethanol and mixed with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). After 90 min incubation at 90°C, the absorbance was measured at 695 nm. The total antioxidant activity was expressed as ascorbic acid equivalent. A blank containing 1 ml reagent and 100 µl distilled water was prepared under the same conditions.

Reducing power capacity

The reducing power capacity of SEOS was determined as previously described,[22] with slight modifications. Briefly, 1.25 ml phosphate buffer (0.2 M, pH 6.6) and 1.25 ml potassium ferricyanide (1% in water, w/v) were mixed with 0.5 ml SEOS (from 2.5 mg/ml to 25 mg/ml). After 20 min incubation at 50°C, 1 ml trichloroacetic acid (10% in water, w/v) was added to the reaction mixture. After centrifugation for 10 min at 3000 rpm, 1.5 ml were taken from the supernatant, then mixed with 1.5 ml distilled water and 100 µl fresh ferric trichloride (0.1% in water, w/v). The reducing power capacity was determined by measuring the absorbance at 700 nm against a blank (SEOS free). A higher absorbance is relevant of high reducing capacity. The ascorbic acid (ranging from 0.01 mg and 25 mg) was used as reference.

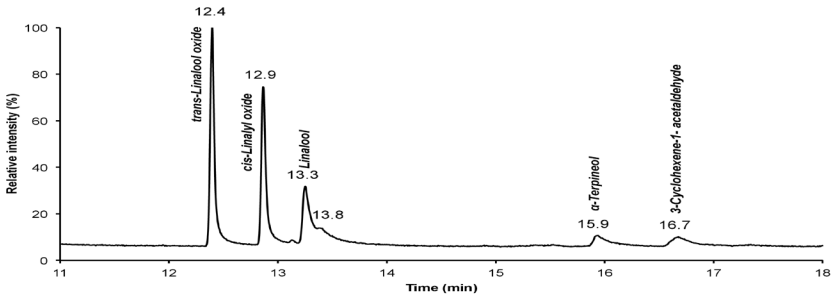


Figure 2: Gas chromatography–mass spectrometry profile of the extracted non-polar compounds from *Opuntia stricta* Haw. fruit peels

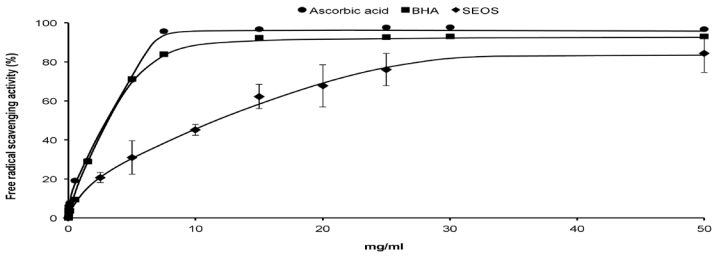


Figure 3: DPPH free radical scavenging activities of butylated hydroxyanisole (BHA), ascorbic acid, and solvent extract from *Opuntia stricta* Haw. fruit peels (SEOS)

Antibacterial activities

The antibacterial activities of SEOS were determined using the disc's diffusion method.[18,23,24] Both Gram positive and negative bacteria were tested. In total, eleven strains were used: *Escherichia coli* (ATCC 25922), *Salmonella typhimurium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus thuringiensis*, *Pseudomonas aeruginosa* (ATCC 27853), *Micrococcus luteus* (ATCC 4698), *Klebsiella pneumoniae* (ATCC 13883), *Enterobacter sp* and *Actinomyces sp*. Solid Luria Broth media (yeast extract; 5 g/l, NaCl; 10 g/l, tryptone; 10 g/l, and agar agar; 18 g/l) was prepared and inoculated with 100 µl (10^6 UFC/ml) of each strain. Four sterile Whatman paper discs (6 mm) were placed on each plate; 2 of them were tested with 15 mg and 30 mg SEOS, respectively, the third one was used as negative control (20 µl hexane) and the last one was used as positive control with either 0.25 mg ampicillin (*E. coli*, *B. subtilis*, *S. aureus*, *E. faecalis*, *M. luteus*, *E. sp* and *A. sp*.) or 0.25 mg ciprofloxacin (*B. thuringiensis*, *K. pneumoniae*, *S. typhimurium*, *P. aeruginosa*). After 18 h incubation at 37°C, the antibacterial activities were measured as the diameter (in mm) of the clear zone of growth inhibition as described previously.[18]

Statistical analysis

The obtained results were expressed as the average of three biological replicates ± standard deviation. Significant differences were determined at 95% confidence by Duncan's multiple range test, using SPSS software 17.0.

RESULTS AND DISCUSSION

Solvent extract composition

The yield of solvent extract obtained by hydrodistillation of *O. stricta* Haw. fruit peels was 0.18% ± 0.06% (w/w). Although the low extraction yield of SEOS, this yield was higher than that found for other *Opuntia* species. In fact, for *O. acanthocarpa*; this yield was only 0.05% and 0.07% for major and discata varieties, respectively.[8] The GC-MS analysis of SEOS revealed the presence of 5

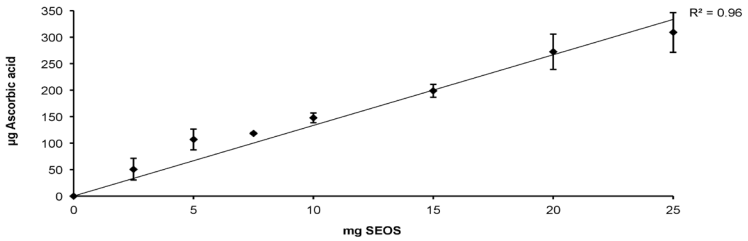


Figure 4: Total antioxidant activity of the non-polar solvent extract from *Opuntia stricta* Haw. fruit peels (SEOS), expressed as µg of ascorbic acid equivalent

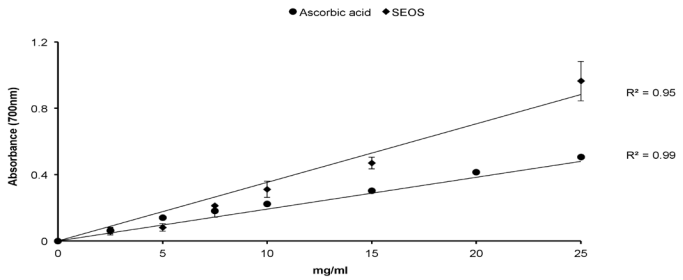


Figure 5: Reducing power capacity of non-polar solvent extract from *Opuntia stricta* Haw. fruit peels (SEOS) compared to ascorbic acid

components (Figure 2); which were identified according to the Wiley library associated with the GC-MS software. The most abundant compounds were *trans*-linalool oxide, *cis*-linalyl oxide and linalool with 38.3%, 29.6% and 23.4%, respectively. Similar compounds were found by Wright and Setzer (2014); who demonstrated that *cis*-linalool oxide and *trans*-linalool oxide were the most abundant components in *O. littoralis* cladodes, with 10.8% and 8.8%, respectively.[8] The presence of linalool at the post-flowering phase of *O. stricta* flowers, was previously reported by Ammar and co-workers (2012), using hexane extract.[25]

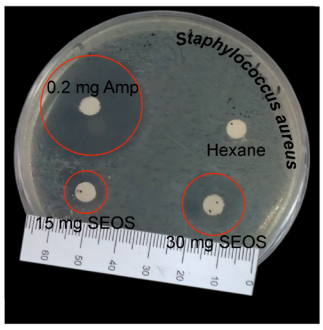


Figure 6: Growth inhibition of *Staphylococcus aureus* using the non-polar solvent extract from *Opuntia stricta* Haw. fruit peels (SEOS). Amp; Ampicillin

Antioxidant activities

DPPH free radical-scavenging activity

Scavenging of DPPH free radicals is the basis of a common antioxidant assay.[26] The DPPH free radical scavenging activities of SEOS, BHA and ascorbic acid are shown in Figure 3. The ability to scavenge the DPPH free radicals increased proportionally to the sample's concentration. The obtained results (Figure 3) showed the potent scavenging activity of SEOS compared to the reference curves (BHA and ascorbic acid). In fact, it inhibits 84% DPPH free radicals at 50 mg/ml concentration. SEOS represents thus a strong electron donor and could react with free radicals to convert them to more stable products and terminate the radical chain reaction.

Total antioxidant activity

The total antioxidant activity was followed by the reduction of the phosphomolybdate by SEOS, determined by measuring the absorbance at 695 nm, and was expressed as ascorbic acid equivalent (AAE) (Figure 4). The obtained results showed a linear behavior between SEOS and ascorbic acid, with correlation coefficient R^2 of 0.96. In fact, the antioxidant activity increases proportionally to the concentration of SEOS. 25 mg of SEOS were equivalent to 309 ± 37 µg ascorbic acid, in terms of total antioxidant activity.

Reducing power capacity

The reducing power capacity reflects the presence of an antioxidant for the reduction of ferricyanide ions $[Fe(CN)_6]^{3-}$ to ferrocyanide ions $[Fe(CN)_6]^{4-}$. This reducing property is generally associated with the presence of a reducer exercising an antioxidant action by breaking the free radical chains; yielding an hydrogen atom.[27]

The reducing power capacity of SEOS was determined by measuring the formation of Perl's Prussian blue at 700 nm (Figure 5). The reducing power of SEOS was increasing proportionally to the sample's concentration and was even higher than that observed for Ascorbic Acid (AA). In fact, at 25 mg/ml, the measured values at 700 nm were 0.96 ± 0.12 and 0.50 ± 0.002 for SEOS and AA, respectively. The correlation coefficients R^2 were 0.95 and 0.99 for SEOS and AA, respectively. These results reveal the high reducing power capacity of SEOS.

The obtained results through these antioxidant activities concur with several works describing the antioxidant activities of *Opuntia* extracts.[16,17,28–32]

Antibacterial activities

The antibacterial activities of SEOS were tested against 11 pathogenic strains as described in the materials and methods section. The obtained results show that the most prominent activity was found against *Staphylococcus aureus* (Figure 6). Partial inhibition was found for *Enterococcus faecalis* and no activity was noticed for the remaining strains. This result is most probably related to the composition of the non-polar extract from *O. stricta* fruit peels. In fact, it has been reported that the extracts composed of either aldehydes or phenols showed the highest antibacterial activities, followed by those containing terpene alcohols.[33] According to the GC-MS profile (Figure 2), SEOS is mainly composed of terpene alcohols, demonstrating thus the low antibacterial activities against the tested strains; remaining although interesting against *Staphylococcus aureus*.

CONCLUSION

Non-polar compounds extracted from *O. stricta* fruit peels, meaning hydrodistillation, showed high antioxidant activities through DPPH free radical scavenging activity, total antioxidant activity and reducing power capacity. The antibacterial activities against 11 pathogenic strains showed a good inhibition only against *Staphylococcus aureus*. These results undertaken together show that *O. stricta* peels are promising feedstock for phytochemical compounds, and extracting these non-polar compounds constitutes a way among others for its valorization.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

This work was funded by the Ministry of Higher Education and Scientific Research of Tunisia.

CONTRIBUTION DETAILS

The first and second authors contributed to the sample's collection, experimental studies and drafting the paper, the third and fourth authors contributed to the manuscript preparation and its revision, the fifth and sixth authors contributed to the concept, design, definition of intellectual content and review the manuscript.

ABBREVIATION

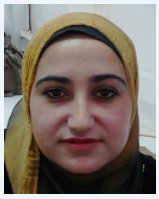
SEOS : solvent extract from *Opuntia stricta*
GC-MS: gas chromatography-mass spectrometry
BHA : butylated hydroxyanisole
AA : Ascorbic acid
DPPH : 2,2-diphenyl-1-picrylhydrazyl

Highlights of the paper

- Solvent extract from *Opuntia stricta* fruit peels (SEOS) contains *trans*-linalool oxide, *cis*-linalyl oxide and linalool with 38.3%, 29.6% and 23.4%, respectively.
- SEOS exhibits high antioxidant activities (DPPH free radical scavenging activities, total antioxidant activity, and reducing power capacity).
- The antibacterial activities of SEOS showed high growth inhibition against *Staphylococcus aureus* and partial inhibition against *Enterococcus faecalis*.

About Authors

Dr. Mohamed Koubaa:*currently apost-doctoral researcher at the department of Industrial Engineering Process, University of Tehnology of Compiègne, France. Before his stay in France, he was working as a post-doctoral researcher at the Ohio State University, USA, then as a researcher at the National Engineering School of Sfax, Tunisia. His field of expertise is mainly food science and technology with an outstanding skills on metabolic engineering and lipid synthesis in plants.*



Ameni Ktata: *currently a PhD scholar at the department of biological engineering, National Engineering School of Sfax, Tunisia. Her work is mainly focused on the extraction and characterization of polysaccharides.*

Fatma Bouaziz: *currently a PhD scholar at the department of biological engineering, National Engineering School of Sfax, Tunisia. Her work is mainly focused on the valorization of almond gum (stucture, phytochemical properties…), with several published papers on this topic.*

Dr. Dorra Driss: *currently a post-doctoral researcher at the department of biological engineering, National Engineering School of Sfax, Tunisia. Her PhD was focused on the study of xylanolitic system of Penicillium occitanis Pol6: purification, biochemical characterisation, heterelogous expression and biotechnological applications. She has published several papers on that field.*

Dr. Raoudha Ellouz Ghorbel: *is an assistant professor at the National Engineering School of Sfax, Tunisia. She has an outstanding knowledges on food science and technology and published several papers on this field.*

Pr. Semia Ellouz Chaabouni: is a professor at the National Engineering School of Sfax, Tunisia, and the director of the Enzyme Bioconversion Unit (UR13ES74) at the same institution. Her research fields are mainly focused on Food Science and Technology, as well as Enzyme engineering.

REFERENCES

1. Castellar MR, Solano F, Obón JM. Betacyanin and other antioxidants production during growth of *Opuntia stricta* (Haw.) fruits. Plant Foods Hum Nutr Dordr Neth. 2012; 67(4): 337-43.
2. Oskoueian E, Abdullah N, Hendra R, Karimi E. Bioactive compounds, antioxidant, xanthine oxidase inhibitory, tyrosinase inhibitory and anti-inflammatory activities of selected agro-industrial by-products. Int J Mol Sci. 2011; 12(12): 8610-25.
3. Martinez-Avila GCG, Aguilera AF, Saucedo S, Rojas R, Rodriguez R, Aguilar CN. Fruit wastes fermentation for phenolic antioxidants production and their application in manufacture of edible coatings and films. Crit Rev Food Sci Nutr. 2014; 54(3): 303-11.
4. Sila A, Bayar N, Ghazala I, Bougatef A, Ellouz-Ghorbel R, Ellouz-Chaabouni S. Water-soluble polysaccharides from agro-industrial by-products: functional and biological properties. Int J Biol Macromol. 2014; 69: 236-43.
5. Ramadan MF, Mörsel J-T. Recovered lipids from prickly pear [*Opuntia ficus-indica* (L.) Mill] peel: a good source of polyunsaturated fatty acids, natural antioxidant vitamins and sterols. Food Chem. 2003; 83(3): 447-56.
6. Cardador-Martínez A, Jiménez-Martínez C, Sandoval G. Revalorization of cactus pear (*Opuntia spp*.) wastes as a source of antioxidants. Food Sci Technol Camp. 2011; 31(3): 782-8.
7. Jorge AJ, Heliodoro DLGT, Alejandro ZC, Ruth BC, Noe AC. The optimization of phenolic compounds extraction from cactus pear (*Opuntia ficus-indica*) skin in a reflux system using response surface methodology. Asian Pac J Trop Biomed. 2013; 3(6): 436-42.
8. Wright CR, Setzer WN. Volatile Compositions of Two Cactus Species Growing in the Sonoran Desert of Southern Arizona. Am J Essent Oil Nat Prod. 2013; 1(1): 41-7.
9. Bergaoui A, Boughalleb N, Ben Jannet H, Harzallah-Shiric F, El Mahjoub M, Mighri Z. Chemical Composition and Antifungal Activity of Volatiles from Three Opuntia Species Growing in Tunisia. Pak J Biol Sci. 2007; 10(15): 2485-9.
10. Zito P, Sajeva M, Bruno M, Rosselli S, Maggio A, Senatore F. Essential oils composition of two Sicilian cultivars of *Opuntia ficus-indica* (L.) Mill. (*Cactaceae*) fruits (prickly pear). Nat Prod Res. 2013; 27(14): 1305-14.
11. Chahdoura H, Barreira JCM, Barros L, Santos-Buelga C, Ferreira ICFR, Achour L. Phytochemical characterization and antioxidant activity of the cladodes of *Opuntia macrorhiza* (Engelm.) and *Opuntia microdasys* (Lehm.). Food Funct. 2014; 5(9): 2129-36.
12. Umar MI, Javeed A, Ashraf M, *et al*. Polarity-Based Solvents Extraction of *Opuntia dillenii* and *Zingiber officinale* for *In Vitro* Antimicrobial Activities. Int J Food Prop. 2013; 16(1): 114-24.
13. Abou Elella FM, Farouk Mohammed Ali R. Antioxidant and Anticancer Activities of Different Constituents Extracted from Egyptian Prickly Pear Cactus (*Opuntia ficus-indica*) Peel. Biochem Anal Biochem. 2014; 3(158): 1009-2161.
14. Kim J, Jho KH, Choi YH, Nam S-Y. Chemopreventive effect of cactus (*Opuntia humifusa*) extracts: radical scavenging activity, pro-apoptosis, and anti-inflammatory effect in human colon (SW480) and breast cancer (MCF7) cells. Food Funct 2013; 4(5): 681-8.
15. Kunyanga CN, Vellingiri V, Imungi KJ. Nutritional quality, phytochemical composition and health protective effects of an under-utilized prickly cactus fruit (*Opuntia stricta* Haw.) collected from Kenya. Afr J Food Agric Nutr Dev. 2015; 14(7): 9561-77.
16. Yeddes N, Chérif JK, Guyot S, Sotin H, Ayadi MT. Comparative study of antioxidant power, polyphenols, flavonoids and betacyanins of the peel and pulp of three Tunisian *opuntia* forms. Antioxidants 2013; 2(2): 37-51.

17. Obón JM, Castellar MR, Alacid M, Fernández-López JA. Production of a red–purple food colorant from *Opuntia stricta* fruits by spray drying and its application in food model systems. J Food Eng. 2009; 90(4): 471-9.
18. Koubaa M, Driss D, Bouaziz F, Ellouz Ghorbel R, Ellouz Chaabouni S. Antioxidant and antimicrobial activities of solvent extract obtained from rocket (*Eruca sativa* L.) flowers. Free Radic Antioxid. 2015; 5(1): 29-34.
19. Yadegarinia D, Gachkar L, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I. Biochemical activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. essential oils. Phytochemistry 2006; 7(12): 1249-55.
20. Saleh MA, Clark S, Woodard B, Deolu-Sobogun SA. Antioxidant and free radical scavenging activities of essential oils. Ethn Dis. 2010; 20(1 Suppl 1): S1-78-82.
21. Bersuder P, Hole M, Smith G. Antioxidants from a heated histidine-glucose model system. I: Investigation of the antioxidant role of histidine and isolation of antioxidants by high-performance liquid chromatography. J Am Oil Chem Soc. 1998; 75(2): 181-7.
22. Yildirim A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. J Agric Food Chem. 2001; 49(8): 4083-9.
23. Celiktas OY, Kocabas EEH, Bedir E, Sukan FV, Ozek T, Baser KHC. Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis***,** depending on location and seasonal variations. Food Chem. 2007; 100(2): 553-9.
24. Sacchetti G, Maietti S, Muzzoli M, *et al*. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem. 2005; 91(4): 621-32.
25. Ammar I, Ennouri M, Khemakhem B, Yangui T, Attia H. Variation in chemical composition and biological activities of two species of Opuntia flowers at four stages of flowering. Ind Crops Prod. 2012; 37(1): 34-40.
26. Sharma OP, Bhat TK. DPPH antioxidant assay revisited. Food Chem. 2009; 113(4): 1202-5.
27. Gordon MH. The Mechanism of Antioxidant Action *in vitro*. In: Hudson BJF, ed. Food Antioxidants. Elsevier Applied Food Science Series. Springer Netherlands; 1990. 1-18.
28. Butera D, Tesoriere L, Di Gaudio F, *et al*. Antioxidant activities of sicilian prickly pear (*Opuntia ficus indica*) fruit extracts and reducing properties of its betalains: betanin and indicaxanthin. J Agric Food Chem. 2002; 50(23): 6895-901.
29. Kuti JO. Antioxidant compounds from four *Opuntia cactus* pear fruit varieties. Food Chem. 2004; 85(4): 527-33.
30. Tesoriere L, Butera D, Pintaudi AM, Allegra M, Livrea MA. Supplementation with cactus pear (*Opuntia ficus-indica*) fruit decreases oxidative stress in healthy humans: a comparative study with vitamin C. Am J Clin Nutr. 2004; 80(2): 391-5.
31. Osorio-Esquivel O, Alicia-Ortiz-Moreno, Álvarez VB, -Álvarez L, Giusti MM. Phenolics, betacyanins and antioxidant activity in *Opuntia joconostle* fruits. Food Res Int. 2011; 44(7): 2160-8.
32. Cha M-N, Jun H-I, Lee W-J, Kim M-J, Kim M-K, Kim Y-S. Chemical composition and antioxidant activity of Korean cactus (*Opuntia humifusa*) fruit. Food Sci Biotechnol. 2013; 22(2): 523-9.
33. Naveed R, Hussain I, Tawab A, *et al*. Antimicrobial activity of the bioactive components of essential oils from Pakistani spices against *Salmonella* and other multi-drug resistant bacteria. BMC Complement Altern Med. 2013; 13(1): 265.